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EFFECT OF PEAT MOSS AND SAND ON ROOTING RESPONSE OF CUTTINGS[1]

A. E. HITCHCOCK

(WITH PLATES V–VII AND FIVE FIGURES)

Introduction

Studies relating to growth responses in plants have been conducted by numerous investigators over a period of many years. As a result of these investigations, many fundamental facts have been established regarding relationships between the plant and its environment. Similar relationships, however, have not been established for cuttings. For example, it is not known definitely to what extent external factors in a rooting medium may modify the tendency to initiation and growth of roots by cuttings. The fact that sand has been universally employed as a medium in which to root cuttings indicates that it has been generally thought that aeration and aseptic conditions are more important for the production of roots by cuttings than nutrient elements such as are present in soil.

MCCALLUM (14) attempted to prove that environmental influences cannot be regarded as directly causing the adventitious formation of roots or shoots. Light, gravity, and moisture were tested. Portions of the cutting were exposed to partially or completely saturated atmospheres as well as to water. *Phaseolus multiflorus* was mainly used for these experiments, although it was stated that *Salix*, *Helianthus*, *Taraxacum*, and *Tolmiea* responded in essentially the same manner. MCCALLUM concluded that regeneration of roots and shoots is determined by the same internal factors which control the devel-

[1] Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

opment of dormant buds. He regarded regeneration as inseparable from growth, and much of his work was concerned with the development of dormant shoot buds. His results do not adequately present or characterize the effect of external conditions on rooting response in a solid medium. So far as polarity is concerned, McCALLUM confirmed the work of VÖCHTING.

VÖCHTING (24) considered polarity of roots and shoots to be due mainly to properties inherent in living matter, although he states that light and moisture may have modifying effects. These conclusions reaffirm the results obtained in earlier experiments. His results show, in addition, that the roots from cuttings in sand were more numerous, larger in diameter, and longer than those from similar cuttings placed in water.

CURTIS (7) reported stimulation of root growth as a result of certain chemical treatments of cuttings prior to their placement in sand. The greatest stimulatory effect on root growth was produced by the use of a 1–2 per cent solution of potassium permanganate. Consistent results were obtained for this treatment in many tests on *Ligustrum ovalifolium*. Increased growth was not obtained, however, when the chemical was added to the rooting medium. Crone's and Knop's nutrient solutions in concentrations of 0.01, 0.10, and 0.50 per cent were found to be injurious to hard wood cuttings of *Ligustrum ovalifolium*.

SMALL (19) tested the effect of adding dilute acetic acid (1:10,000) to coconut fiber and to soil, and reported an increased percentage of rooting, or a reduction in time required for rooting, for all treated lots of cuttings as compared with the untreated ones. This treatment was found to be favorable for many varieties of cuttings. On the other hand, VIERHELLER (23) failed to improve the rooting of apple cuttings when SMALL'S acid treatment was used. PHILLIPS (17) likewise obtained no marked improvement in rooting when dilute solutions of acetic acid were added to sand, to soil, or to a synthetic leaf mold, in which cuttings of *Ocotea bullata* were placed. It would appear from these results that all kinds of cuttings will not respond in the same way to a given chemical modification of the rooting medium. PHILLIPS found, however, that when etiolated shoots of *Ocotea* were used for cuttings instead of normal shoots, a more favorable rooting response was obtained. Tests in sand, clay,

and synthetic leaf mold gave, in the order named, 30, 20, and 18 per cent rooting as compared with 6 per cent for the best lot of cuttings made from normal shoots. The physiological condition of the material selected for cuttings, therefore, is seen to be of considerable importance when comparing their ability to root in different media.

ZIMMERMAN (25) stated that peat moss was a good medium in which to root *Ilex*, Delaware grape, and some *Viburnum* cuttings. The last usually rooted at the base in sand, whereas in a peat moss medium rooting took place all along the portion of the stem which was buried. It was suggested that peat moss may contain stimulating substances which cause this specific response. The same worker also pointed out that favorable results might be expected with mixtures of peat moss and sand. These mixtures, it was stated, would hold water better than sand, and some benefit might be expected from the presence of peat moss.

SMITH (20) reported on the relation of acidity of the medium and root production in *Coleus*. Although *Coleus* cuttings rooted very readily in coconut fiber (pH 4.5–4.7), it was found that in a series of acid-alkali solutions ranging from pH 4.0 to 9.0, best rooting occurred at pH 7.0. In these solutions there appeared to be a direct relation between pH value and rooting response, if the dry weight of roots per gram of top dry matter was taken as the criterion of growth. SMITH inferred that the favorable rooting in acid coconut fiber was due to the efficient aeration provided by this medium. This conclusion was arrived at from the fact that the bases of cuttings in the fiber gave an immediate fat test with Sudan III, in contrast to a practically negative test for cuttings rooted in solutions. The presence of fatty substances at the base of the cutting was regarded as due to a plentiful supply of oxygen.

A preliminary report has been published on variation in rooting response of cuttings placed in media of different pH values (10). It was pointed out that *Azalea amoena* cuttings rooted well in natural peat moss, but rooted poorly in sand and in neutral peat moss. Cuttings of California privet gave the opposite response in these media. In a mixture composed of equal parts of peat moss and sand, both *Azalea* and privet rooted exceptionally well, indicating that such a mixture might prove favorable for many varieties of cuttings which root poorly in either peat moss or in sand.

KNIGHT and WITT (12) found that for rooting plum cuttings sand was more suitable than mixtures of coconut fiber and loam, loam and sand, or coconut fiber and sand. In these mixtures there was considerable variation in rooting for the four varieties tested, but in only one case was the percentage of rooting as high as that in sand.

The published data show clearly that root production by cuttings of one variety may be very different from that by cuttings of another variety, when both are placed in the same medium. Only a few varieties have been tested by any single worker, however, and comparable data by different workers are available for only a very small number of plant forms. It is evident, then, that before any generalizations can be made regarding the conditions which should obtain in a rooting medium in order to give favorable rooting, many different kinds of plants must be tested. Owing to the need for more specific information relating to this general subject, an experimental study of vegetative propagation was begun in 1925 at the Boyce Thompson Institute for Plant Research in Yonkers, New York. The purpose of this project is to determine, so far as possible, the important factors concerned in propagating plants vegetatively. Many phases of the project are now being studied. This paper reports the findings relating to the effect of peat moss and sand on rooting response of stem cuttings.

An attempt has been made by the writer to cover a wide range of plant types in making these media tests of cuttings. In the experiments reported 91 varieties of plants, including 46 genera, were used. In addition to obtaining data on differences in rooting response for many varieties of cuttings, an attempt has been made to determine to what extent the acid reaction of peat moss is concerned in producing favorable or unfavorable conditions for root production. The results here recorded are based on work which was done over a period of three years.

Materials and methods

Peat moss, sand, and mixtures of these two constituted the media used in the experiments.[2] Due to the marked contrast in their physi-

[2] Granulated peat moss was obtained from Atkins and Durbrow, New York City. Sand was obtained from the Yonkers Builders Supply Co., Yonkers, N.Y. The sand is reported to come from Cow Bay, Long Island, N.Y.

cal and chemical properties, as shown in table I, peat moss and sand afford unusually good means for obtaining widely divergent conditions without at the same time introducing more than two sets of substratum complexes.

Other phases of the work have shown that the capacity of cuttings to form roots is dependent to some extent upon the age of the wood, season taken, activity of growth, and the amount of leaf surface left on the cutting. These factors were accordingly taken into consideration when selecting material for the experiments. All leafy cuttings were brought into a turgid state, before placement in the rooting medium, by soaking in water for a few minutes or longer if necessary. In the greenhouse, pots were used in most cases to hold

TABLE I

PROPERTIES OF PEAT MOSS COMPARED WITH THOSE OF BUILDING SAND

PROPERTY	PEAT MOSS	SAND
Reaction (pH value)	3.6	7.0+
Wt. of water in gm. held by 100 gm. dry weight peat moss	1000*	20
Origin	Sphagnum bogs	Rock strata
Organic matter content	09 per cent	Negligible
Texture	Finely fibrous	Coarse crystalline
Weight in gm. of 100 cc. (air-dry)	15†	162

* DACHNOWSKI gives a value of 1635.5.

† The moisture content of peat moss in the bale will vary greatly according to the humidity conditions under which it is stored.

the media; outside in the cold frames, cuttings were placed in beds of media protected by an overhead slat shade, no sash being used.

Since sand dries out readily when not covered by sash, an auto-irrigator was used in some of the experiments to provide the rooting medium with a higher and a more nearly constant moisture supply. The auto-irrigator consisted of a small clay pot placed inside of a larger pot, with sand or other media between the walls of the two pots. By keeping a supply of water in the smaller pot, the medium was assured of a continuous and a comparatively constant moisture supply. It was necessary for this purpose to select pots which lost water readily.

All hydrogen-ion measurements were made with a quinhydrone electrode apparatus of a type devised by W. J. YOUDEN, of the Boyce

Thompson Institute for Plant Research. Samples of the media were prepared by adding a sufficient amount of tap water to produce a few cubic centimeters of free liquid. These were allowed to stand overnight in stoppered flasks. A portion of the sample was then transferred to the electrode chamber by means of a small porcelain spoon. The sample thus measured, in the case of peat moss, was of the consistency of mush. Tap water was used, since preliminary tests showed there was no difference between the values obtained for samples made up with tap water and those made up with distilled water. On duplicate samples of standard buffer solutions the accuracy of the particular quinhydrone apparatus used by the writer was found to be within pH 0.03. When checked with a standard hydrogen apparatus, the agreement was within pH 0.03. A standard calomel half-cell was always used in making the measurements with the quinhydrone apparatus.

The reasons for using a heavy suspension of peat moss rather than an extract are explained in connection with the results showing the buffer capacity of peat moss. A heavy suspension appears to be more representative of the actual conditions in the rooting medium. Although OLSEN and LINDERSTRØM-LANG (16) found a filtrate from soil samples to be less variable than a suspension, they recommend using a minimum of water in making up samples for hydrogen-ion determinations. DOMONTOVITSCH (9) found that many plant juices give the same pH values regardless of whether or not the suspended particles were removed. Tomato leaves, however, gave higher pH values when the centrifuged extract was used than when the original extract was used. In comparing the hydrogen electrode with the quinhydrone electrode for measuring the pH values of plant juices, DOMONTOVITSCH found reasonably good agreement except for turnip and onion, in which cases there were appreciable differences in the readings.

BRAY (3) found that if the particles of a soil suspension were agitated by blowing in hydrogen gas from the bottom of the electrode chamber, the pH value was different from that obtained when the particles were allowed to settle out in the bottom of the vessel. These recent investigations have been particularly concerned with the part which solid materials play in affecting the measurement of

hydrogen-ions by the electrometric method. The tendency appears to be toward the use of suspensions rather than filtrates or extracts.

The time at which a reading should be taken after the quinhydrone is added to the sample will no doubt vary according to the type of material being tested. This applies particularly to suspensions. CRUZ (6) took readings at the end of one minute. SNYDER (21) recommends from one-half to one minute for taking readings of the pH. Neither states why these time periods were chosen. As will be shown by the results obtained for buffer tests, an immediate equilibrium is not reached when quinhydrone is added to a heavy suspension of peat moss.

Experimental results

A general classification of rooting response for 96 varieties of cuttings is given in table II. Experiments recorded in tables III, IV, and V show the degree of rooting which was characteristic for each of the three groups listed in table III.

Experiment 1 (table III) shows that *Azalea amoena* cuttings rooted readily in natural peat moss and in a mixture of peat moss and sand, but that in neutral sand, neutral peat moss, and in a mixture of sand and neutral peat moss much poorer rooting was obtained. Eleven other experiments with *Azalea amoena* gave essentially the same results. Experiment 2 (table III) is mentioned primarily to show that neutral peat moss which has been previously used has different effects from those of a freshly prepared lot of the same material. Although *Azalea ledifolia* (*indica alba*) does not root so readily or so uniformly as *Azalea amoena*, the relative differences in the amount of roots formed in different media were found to be essentially the same. The difference in the size of the root systems for *Azalea amoena* cuttings rooted in peat moss and those rooted in sand is shown in figs. 1, 2. Uniformity of rooting in peat moss and lack of uniformity in sand are also evident in the same figures.

Prunus glandulosa rooted poorly in peat moss, exhibiting an injury similar to that of privet cuttings in the same medium. Excellent root systems were produced in sand, however, and in a mixture of sand and peat moss. These results are recorded in table IV (experiments 5 and 6). The results recorded for experiment 5 are also

TABLE II

CLASSIFICATION OF VARIETIES OF CUTTINGS ACCORDING TO ROOTING RESPONSE IN PEAT MOSS AND SAND

GROUP I CUTTINGS ROOTED READILY IN PEAT MOSS BUT POORLY IN SAND	GROUP II CUTTINGS ROOTED READILY IN SAND BUT POORLY IN PEAT MOSS	GROUP III CUTTINGS ROOTED READILY IN EITHER PEAT MOSS OR IN SAND	
Azalea amoena* (6–12)†	Asclepias nivea (1–4)	Buddleia davidii (8)	Ribes alpinum (6–8)
Azalea ledifolia* (8)	Berberis thunbergi (8)	Buxus sempervirens (7, 11)	Ribes nigra (6–8)
Azalea (hardy mollis hybrids) (7)	Carnation (7)	Callicarpa purpurea (6–8)	Rosa (American Pillar) (6–9)
Blue spruce (2–4)	Cotoneaster horizontalis (7)	Capsicum grossum (4)	Rosa (Dorothy Perkins) (6–9)
Delaware grape (7)	Daphne cneorum (7)	Coleus blumei (4 var.)* (1–12)	Rosa setigera (5)
Enkianthus campanulatus (9)	Datura stramonium (1)	Cornus florida (pink) (6, 7)	Rosa (Silver Moon) (7)
Ilex crenata (3)	Deutzia gracilis* (6–8)	Cornus florida (white) (6, 7)	Salix alba (5, 6, 11)
Vaccinium corymbosum (6–8)	Heliotrope (10)	Cornus kousa (6)	Salvia splendens (5, 8)
	Lagerstroemia indica (8)	Cornus mas (6)	Sambucus canadensis (6–8)
	Ligustrum ibota (var. regelianum)* (8)	Dahlia (6 var.) (4, 5, 8–11)	Spiraea (Anthony Waterer) (6–8)
	Ligustrum japonicum (9)	Evonymus radicans (7, 8)	Spiraea arguta (6–8)
	Ligustrum ovalifolium* (6, 8, 11)	Evonymus (variegated) (7)	Spiraea nipponica rotundifolia (6–8)
	Ligustrum vulgare* (8)	Forsythia intermedia (5–9)	Spiraea reevissiana (6–8)
	Mentha piperita* (7–10)	Forsythia viridissima (5–9)	Spiraea thunbergii (6–8)
	Osmanthus aquifolium (white variegated) (8)	Fuchsia speciosa (3 var.) (6–9)	Spiraea van houttei (6–8)
	Prunus glandulosa (pink variety)* (6–8)	Gardenia florida (2, 7)	Symphoricarpos racemosus (6–8)
	Prunus tomentosa* (6)	Geranium (4, 6, 10–12)	Symphoricarpos vulgaris (6–9)
	Prunus triloba (12)	Hydrangea opuloides (8)	Taxus cuspidata (1, 6, 11)
	Rosa hugonis (6)	Hydrangea petiolaris (6)	Taxus (weeping variety) (1)
	Syringa vulgaris* (6, 7)	Ilex aquifolium (8–10)	Tsuga canadensis (6–8)
		Ilex cornuta (1)	Ulmus parvifolia (6)
		Ilex glabra (8–12)	Ulmus pumila* (5, 6)
		Ilex opaca (6, 8–12, 1)	Viburnum carlesii (7)
		Lonicera grafravitissima (7, 8)	Viburnum opulus americanum (6–8)
		Lonicera morrowi (7, 8)	Viburnum opulus sterile (6–8)
		Lonicera tatarica (7, 8)	Viburnum tomentosum plicatum (1–3, 12)
		Philadelphus coronarius (6–8)	Weigela floribunda (6–9)
		Philadelphus folconeri (6–8)	Weigela rosea (7)
		Philadelphus gordonianus (6–8)	
		Philadelphus grandiflorus (6–8)	
		Philadelphus leusi (6–8)	

* Cuttings tested in neutral peat moss.
† Numerals in parenthesis indicate months during which cuttings were taken; for example, (6) refers to June.
Note: Cuttings in all three groups rooted readily in a mixture of equal parts of peat moss and sand, with the exception of the five varieties mentioned in the text.

TABLE III

* Cuttings tested in neutral peat moss.

† Numerals in parenthesis indicate months during which cuttings were taken; for example, (6) refers to June.

Note: Cuttings in all three groups rooted readily in a mixture of equal parts of peat moss and sand, with the exception of the five varieties mentioned in the text.

TABLE III

TYPICAL ROOTING RESPONSE FOR CUTTINGS OF VARIETIES LISTED IN GROUP I

No. of experiment	Variety of cutting	Date	No. of cuttings in each medium	Rating of root system in different media									
				Sand		Mixture sand and peat moss		Natural peat moss		Neutral peat moss		Mixture sand and neutral peat moss	
				Percentage rooted	Size root system	Percentage rooted	Size root system	Percentage rooted	Size root system	Percentage rooted	Size root system	Percentage rooted	Size root system
1.....	Azalea amoena	7–11 to 8–16–27	40	60	++*	83	+++	90	+++	37	+	50	+
2.....	Azalea amoena	8–28 to 10–18–27	30	60	++	90	+++	97	+++	76†	+	90†	++
3.....	Azalea ledifolia	8–28 to 10–18–27	30	22	++	60	++	56	+++	67†	++	60†	++
4.....	Ilex crenata	2–28 to 3–28–27	20	55	+	35	+	72	+++	.....	.....	.....	.....

* + represents a poor root system; ++ a fair root system; and +++ a normally vigorous root system.

† Medium which had been used previously and was 3 months old.

TABLE IV

TYPICAL ROOTING RESPONSE FOR CUTTINGS OF VARIETIES LISTED IN GROUP II

No. of experiment	Variety of cutting	Date	No. of cuttings in each medium	Rating of root system in different media									
				Sand		Mixture sand and peat moss		Natural peat moss		Neutral peat moss		Mixture sand and neutral peat moss	
				Percentage rooted	Size root system	Percentage rooted	Size root system	Percentage rooted	Size root system	Percentage rooted	Size root system	Percentage rooted	Size root system
5.....	Prunus glandulosa (pink)	6–25 to 8–10–27	15	100	++*	100	+++	50	+	100	+++	100	+++
6.....	Prunus glandulosa (pink)	6–30 to 8–17–27	50	97	+++	78	+++	24	++	.....	.....	.....	.....
7.....	Deutzia gracilis	6–15 to 8–6–27	20	100	+++	100	++	50	+	.....	.....	.....	.....
8.....	Ligustrum ovalifolium	7–1 to 8–11–27	20	70	++	100	+++	30	+	.....	.....	.....	.....

* + represents a poor root system; ++ a fair root system; and +++ a normally vigorous root system.

TABLE V

TYPICAL ROOTING RESPONSE FOR CUTTINGS OF VARIETIES LISTED IN GROUP III

No. of experiment	Variety of cutting	Date	No. of cuttings in each medium	Rating of root system in different media							
				Sand		Mixture sand and peat moss		Natural peat moss		Neutral peat moss	
				Percentage rooted	Size root system	Percentage rooted	Size root system	Percentage rooted	Size root system	Percentage rooted	Size root system
9.....	Coleus blumei	12–1 to 12–20–26	30	100	+*	100	+	100	+	100	++
10.....	Coleus blumei	9–24 to 10–15–27	10	100	+	100	++	100	+	100	+++
11.....	Viburnum opulus sterile	6–30 to 8–25–27	20	100	+++	100	+++	100	+++	.....	.....
12.....	Cornus florida	6–16 to 7–26–27	15	35	+	83	++	35	+	.....	.....
13.....	Ilex opaca	9–15 to 11–16–26	15	54	++	85	+++	67	++	.....	.....

* + represents a poor root system; ++ a fair root system; and +++ a normally vigorous root system.

shown in fig. 3. It is important to note that the best rooting of almond cuttings was obtained in neutral peat moss and in the mixture of sand and neutral peat moss.

Five varieties of cuttings listed under group II (table II) rooted poorest in peat moss, only slightly better in a mixture of sand and peat moss, and exceptionally well in sand. These varieties were *Deutzia gracilis*, *Prunus tomentosa*, *Daphne cneorum*, *Ligustrum japonicum*, and *Syringa vulgaris*. All other cuttings in group II rooted fully as well in a mixture of sand and peat moss as they did in sand.

Cuttings listed in group III rooted readily in both peat moss and in sand, although in most cases the best rooting occurred in a

TABLE VI

VARIATION IN pH VALUE OF CENTRIFUGED PEAT MOSS EXTRACT DUE TO DIFFERENT METHODS OF RINSING ELECTRODE AND ELECTRODE CHAMBER

RINSED WITH RUNNING TAP WATER (DUPLICATE LOTS)		RINSED WITH TEST SOLUTION	NO RINSING	RINSED WITH DISTILLED WATER
4.36	4.22	3.99	3.94	3.94
4.51	4.49	3.94	3.94	3.94
4.65	4.49	3.94	3.94	3.94
4.41	4.65	3.94	3.94	3.94

mixture of sand and peat moss. Examples of this type are given in table VI. Ten other experiments with *Coleus* showed that in neutral peat moss by far the largest root systems were produced. For *Coleus* cuttings it was immaterial whether peat moss was neutralized by adding calcium carbonate, a calcium carbide waste product, an asbestos product, or by leaching with tap water.

A few experiments were conducted for the purpose of determining the effect of the medium on root growth after the roots had appeared. The results for mint cuttings are given in fig. 4. Similar results were obtained for *Coleus*, except that in this case the cuttings were not so sensitive to peat moss before being transferred. The check lots of cuttings were removed from the medium and replaced at the same time that duplicate lots were transferred from one kind of medium to another. These results show that the rate of root

growth in *Coleus* and mint cuttings is retarded by natural peat moss and accelerated by neutral peat moss.

Results of tests in which an auto-irrigator was used to supply moisture showed that root production could be improved by this

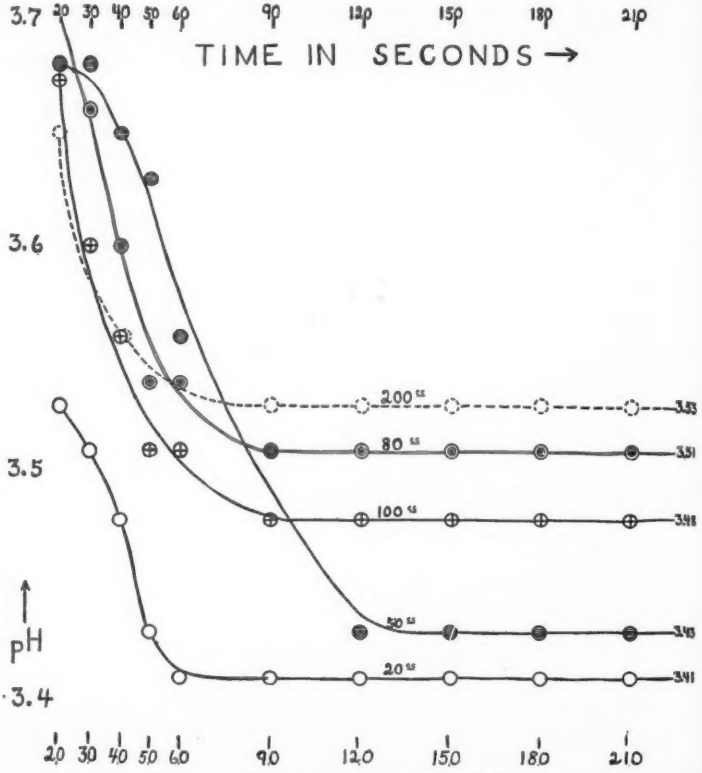

FIG. 7.—Equilibrium relations in electrode chamber for heavy suspension of peat moss, showing change in pH value with time at which readings were taken.

method, especially under conditions of high light intensity. The following cuttings were subjected to the auto-irrigator treatment: privet (3 varieties), *Prunus tomentosa*, Pillar rose, Dorothy Perkins rose, heliotrope, *Deutzia gracilis*, *Spiraea*, *Philadelphus*, *Asclepias*, and *Ilex opaca*. The results for two varieties of privet and for *Prunus tomentosa* are shown in figs. 5 and 6.

Buffer properties of peat moss

Difficulties first met with in measuring the hydrogen-ion concentration of peat moss made it necessary to learn more about the buffer capacity of this material. The data in fig. 7 show the variation in pH value that results when readings are made immediately after quinhydrone is added to a heavy suspension of peat moss in

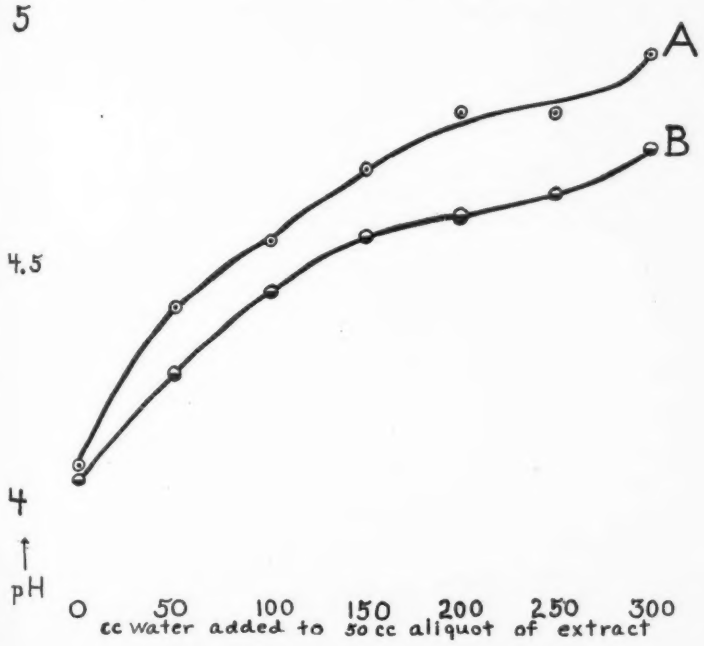

Fig. 8.—Change in pH of peat moss extract due to dilution

the electrode chamber. The change in all cases was in the same direction, that is, toward a more acid value. An equilibrium was practically reached at the end of one minute. Dilution values on each curve represent the amount of water added to 5 gm. of air-dry peat moss. For this particular lot the peat moss contained 50 per cent of moisture, although its moisture content in the bale varied from 30 to 150 per cent, according to the humidity conditions under which it was stored. The 20 cc. dilution curve represents the approximate

minimum moisture content at which a pH measurement of peat moss can be made with the quinhydrone apparatus.

Although the addition of water to peat moss, in great excess of that required to make up a sample for hydrogen-ion determination, does not alter appreciably the pH value of a heavy suspension, the dilution of a peat moss extract causes a marked change in pH value. The results for dilution of an extract are shown in fig. 8. Curves *A* and *B* represent dilution values for extracts from two different lots of peat moss. In both cases the extracts were freed from solid par-

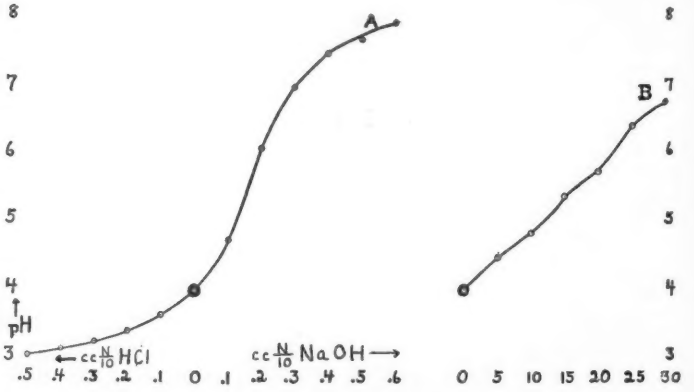

FIG. 9.—*A*, change in pH of peat moss extract due to addition to HCl and NaOH; *B*, change in pH of peat moss due to addition of NaOH.

ticles by centrifuging. These dilution curves show that a water extract is not very efficiently buffered. Further evidence to support this fact is given in fig. 9, which shows the result of adding N/10 sodium hydroxide and N/10 hydrochloric acid to 50 cc. portions of peat moss extract. Curve *A* (fig. 9) represents the change in pH value of a peat moss extract due to the addition of acid and alkali. Since only small amounts of either acid or base were required to cause an appreciable change in pH value, the extract cannot be considered as being efficiently buffered. The effectiveness of the solid material in taking out large quantities of base is shown by the results represented in *B*. In this case fifty times the concentration of base was

used, with the difference that the sodium hydroxide was added to the peat moss 24 hours before an extract was made.

In order to determine whether the capacity of peat moss to take up sodium hydroxide is a quantitative reaction, larger aliquots of peat moss and proportionally greater concentrations of base were used. Curves for these results are shown in fig. 10. *A* represents the change in pH value due to the addition of alkali in 5 cc. incre-

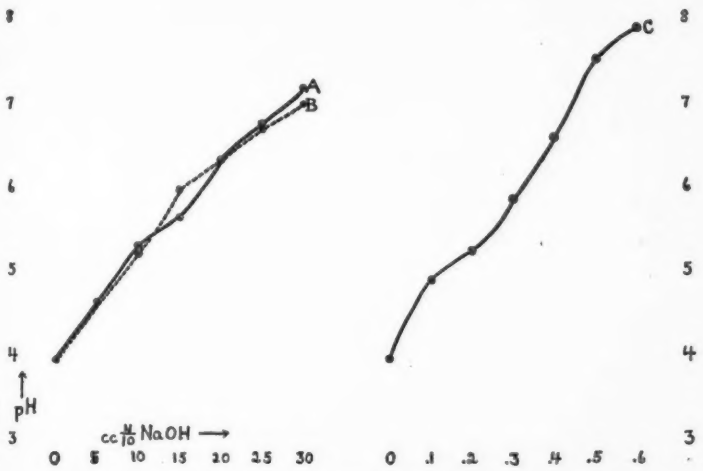

FIG. 10.—*A*, change in pH due to addition of NaOH to 50 cc. portions of peat moss; *B*, change in pH due to addition of four times the amount of NaOH to four times the volume of peat moss as used for obtaining values for *A*; *C*, change in pH of extract from 50 cc. of peat moss due to addition of NaOH.

ments to 50 cc. portions of peat moss. *B* represents the change in pH value due to the addition of alkali in 20 cc. increments to 200 cc. portions of peat moss. In both cases extracts were made 24 hours after alkali was added. *C* represents the change in pH value due to the addition of alkali in 0.10 cc. increments to the extract obtained from 50 cc. of peat moss. Although the same amounts of peat moss were used in obtaining extracts for values in *A* and *C*, the neutralizing capacity (*A*) is shown to be over fifty times that of its extract (*C*).

Since powdered calcium carbonate was usually employed to neutralize peat moss used in media tests, the effect of adding 1 gm. increments to liter portions is shown in fig. 11. *A* and *B* represent the change in pH value for an extract and a heavy suspension respectively, obtained from peat moss to which had been added 1

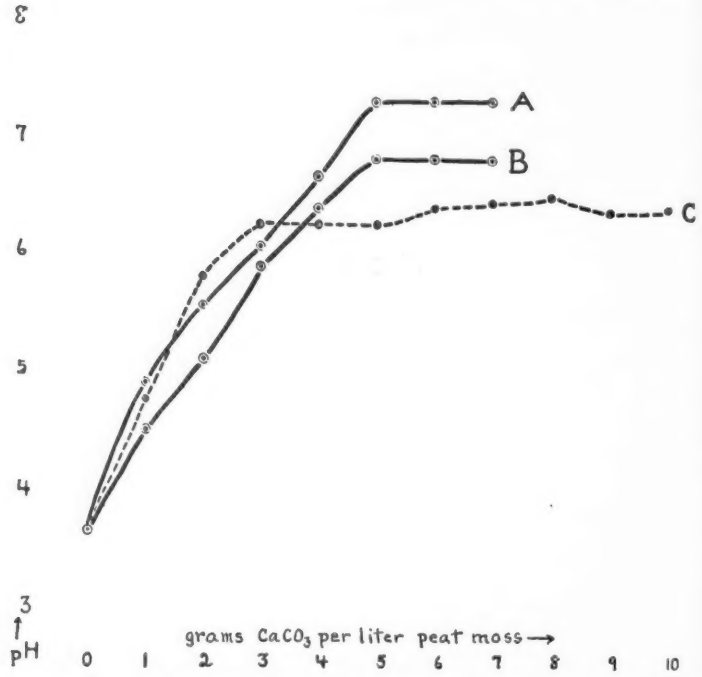

FIG. 11.—Change in pH of peat moss due to addition of increasing amounts of $CaCO_3$.

gm. increments of carbonate. Readings for these two curves were taken 5 days after addition of the carbonate. The results after 2 days for another lot of peat moss are shown in *C*. Higher pH values were invariably obtained for extracts than were obtained for heavy suspensions. This fact held true for both the natural peat moss and for that to which carbonate had been added. The time for complete neutralization with an excess of carbonate depends upon the method

of mixing in the carbonate. Ten grams of carbonate per liter of moistened peat moss will bring about neutralization in 24–48 hours if the material is thoroughly mixed and repeatedly squeezed with the hands. Sufficient water must be added to give the consistency of mush.

When the quinhydrone electrode and the electrode chambers were rinsed with running tap water, an error of considerable proportions was introduced. Rinsing with distilled water or with the test solution caused no error. Likewise, no error was introduced if the solution was completely thrown out of the electrode chamber without any subsequent rinsing procedure. Comparisons of different methods of rinsing are given in table VI.

Discussion of results

Contrary to the general belief that sand is the most suitable medium in which to root most kinds of cuttings, it was found that this held true under the conditions given for only 6 out of 96 varieties tested (table II). On the other hand, a mixture of peat moss and sand proved to be far superior to sand, inasmuch as 90 out of 96 varieties of cuttings rooted readily in this mixture. These results are of particular interest, since the advice in textbooks on propagation and in a recent paper by STEWART (22) is to the effect that "clean sharp sand" be used for rooting most kinds of cuttings.

The classification of cuttings according to their rooting response, as given in table I, applies in some cases only to cuttings taken within a limited period during the year, although most of the cuttings were taken from June 1 to October 1. *Prunus tomentosa* rooted readily when taken from May 15 to June 20, but much poorer rooting occurred after this period. *Cornus florida* cuttings taken in June rooted more readily than those taken at any other time. Maximum rooting for blue spruce occurred from February 15 to April 15. In contrast to the varieties just mentioned, *Azalea amoena* and California privet cuttings rooted at all times of the year. It is readily seen, then, that seasonal variation is of considerable importance in testing the effect of the medium on root production by cuttings.

In most cases species of the same genus responded similarly in a given medium. *Ilex crenata* and *Rosa hugonis* were exceptions to

this rule. Whereas the former rooted best in peat moss and the latter rooted best in sand, four other species of both *Ilex* and *Rosa* rooted best in a mixture of sand and peat moss. *Ilex opaca* taken in the fall appeared to root more readily in peat moss than when taken during December and January. *Daphne cneorum* was particularly sensitive to peat moss when young shoots were used, but with more mature shoots this effect was much less noticeable. Although blue spruce cuttings taken in late winter and early spring rooted best in peat moss, young shoots of the active spring growth were injured in the same medium. Such seasonal variations in rooting make a rigid classification of cuttings practically impossible, unless it be confined to a definite season of the year. Inasmuch as all of the varieties listed in table II were not tried at all times of the year, a complete seasonal classification could not be made.

For those cuttings which rooted best in peat moss, or in a mixture of peat moss and sand, the appearance of roots occurred at an earlier period, and the rate of growth was more rapid than for similar cuttings placed in sand. This relation held true for cuttings of woody plants but not for *Coleus* and mint, in which cases only a difference in rate of root growth was evident. Although in some cases, as for example with *Azalea amoena*, a fairly high percentage of rooting was obtained in the unfavorable medium, there were very marked differences in the size of the root systems, as illustrated in fig. 1.

Uniformity of root production in peat moss, in contrast to lack of uniformity in sand, is an important feature of the results shown in figs. 1, 2. Since conditions favoring rapid evaporation obtained in the greenhouse during the course of the experiment (August 28 to October 18, 1927), lack of uniformity in sand may be accounted for principally by variation in moisture conditions in different lots of the same medium. Variation in rate of water loss from different pots, with possibly unequal amounts of water added, was no doubt the cause of different moisture conditions in similar lots of sand. Peat moss, being such an efficient moisture retainer, does not dry out readily, however, and hence does not reach the critical low moisture content at which drying of cuttings takes place.

In connection with the subject of moisture content of the medium, it is of interest to note the results of tests by KNIGHT and WITT

(12). For fruit tree cuttings placed in soil under outside conditions, these investigators found that the best rooting was correlated with the least amount of rainfall, and that poorest rooting was correlated with the greatest amount of rainfall. These results are contrary to those obtained by the writer when using peat moss, sand, and a mixture of these two media. No doubt the general complexes of conditions are quite different in the two cases and different varieties of cuttings were used, so that a generalization based upon one set of conditions may not hold true for another. In contrast to the results obtained by KNIGHT and WITT for cuttings in soil, the writer found that a high moisture content, furnished by an auto-irrigator, favored root formation in many varieties of cuttings. Soft, succulent cuttings were usually rooted more readily than were more mature cuttings of the same plants. While this method was especially effective for sand, rooting in peat moss was also improved by auto-irrigation, more particularly when excessive evaporation conditions were provided such as are given by high light intensity, low humidity, and a relatively high temperature.

Cuttings which rooted poorly in peat moss usually showed definite signs of injury. Browning of the basal cut surface and sometimes the lower one-fourth of the cutting often occurred. This injury was particularly noticeable in the case of *Ligustrum*, *Syringa*, and *Prunus*. No callus was formed when this type of injury occurred. Partial or complete neutralization of peat moss allowed formation of good callus, and prevented the injury just described. It was necessary to decrease the acidity only slightly, that is, from pH 3.6 to 4.1, in order to prevent this type of injury. Hard wood cuttings of *Ligustrum ovalifolium* and *Syringa vulgaris* taken in the winter did not show this injury, and good callus formation was obtained on these cuttings even in peat moss of a high moisture content. It seems of interest to point out that in peat moss of a low moisture content (140 per cent) good callus formation occurs on many hard wood cuttings. At this low moisture content peat moss feels dry to the touch, yet a reading with a wet-and-dry bulb apparatus indicated a relative humidity of 96 per cent. The wet-and-dry bulb apparatus was buried in the peat moss, and the reading was made after a temperature equilibrium had been reached. Since

cuttings were rooted regularly in peat moss containing 500–800 per cent of moisture, it is readily seen that this medium furnished an unusually peculiar set of moisture conditions.

That the acidity of peat moss cannot be considered as the single controlling factor determining its value as a rooting medium is shown by fig. 3. Inasmuch as similar results were obtained with other cuttings listed in group II (table II), these results appear to be particularly significant. If the pH value of the medium were considered to be the limiting factor, there should be comparable root systems in lots A, D, and E, with lot B intermediate between lot C and lot A. Such responses did not occur. On the other hand, if mechanical texture such as provided by a mixture of sand and peat moss (lot B) were the limiting factor, then neutralization of the mixture should not have given the noticeable improvement shown in lot E.

It is difficult to explain why a difference of pH 0.6, the approximate difference in pH value between peat moss and a mixture of peat moss and sand, should allow such marked difference in rooting as was regularly shown. If it is assumed that the pH value of the mixture (4.1–4.4) is that at which maximum root formation occurs for cuttings listed in group II (table II), then a more favorable response should not occur in neutral media as is shown in lots D and E (figs. 3, 4). Such a complicated situation can be explained only on the basis that a combination of factors is operating to bring about a given type of rooting response. It is evident, however, that the pH value of peat moss is in some cases one of the most important factors, especially in connection with a direct injury to the cuttings, but in some cases affecting the rate of root growth. For cuttings which are not injured, however, the most important factor in peat moss appears to be the substances it contains (other than its acid reaction) which promote and maintain a rapid rate of root growth.

In addition to experiments with cuttings, the buffer tests on peat moss show that this material furnished conditions that are extremely difficult to analyze. Inasmuch as the hydrogen-ion concentration of peat moss varies according to the method of preparing samples for measurement, it is quite likely that the pH value as measured by the quinhydrone electrode, or by other means, does

not represent the actual hydrogen-ion concentration with which the cutting or roots have to contend. Regardless of the errors which may be involved when pH measurements are made on heavy suspensions of peat moss, it would appear that this type of sample is a more representative one than is an extract of the same material. The capacity of peat moss to take up such large quantities of sodium hydroxide, as shown by the titration curves, indicates that a solid medium such as peat moss must furnish a far more complex set of equilibrium relations than does a liquid medium which contains similar solutes.

Some varieties of cuttings rooted best when the reaction of the medium was comparable with that in which the parent plants are known to produce maximum growth. This is particularly true for *Azalea*, blueberry, lilac, and privet. While it is not the purpose of this report to follow out such comparisons, the results obtained indicate that cuttings of many plants will respond in a similar manner.

Peat moss and a mixture of peat moss and sand will leach sufficiently for a change of approximately pH 0.5 to occur during the course of an experiment, that is, in from six to ten weeks. Whereas the initial pH value of peat moss is 3.6, its final pH value at the end of an experiment would be 3.9–4.1. When the same peat moss was used again for privet cuttings, no marked injury resulted of the kind typical for freshly prepared peat moss. This fact further substantiates the idea that the criticial pH value at which cuttings of privet are injured lies more specifically between 3.6 and 4.1. Although *Azalea* cuttings rooted readily in this type of "used" peat moss, root growth on privet cuttings was not so good as that obtained in a mixture, or in neutral peat moss. As was pointed out in experiments 2 and 3 (table III), *Azalea* cuttings rooted much better in a mixture of neutral peat moss and sand which had been previously used, than in a freshly prepared mixture. *Rhododendron* cuttings (*R. maximum*) formed excellent root systems in a leached peat moss which had previously been used in other experiments. The reaction of this medium four months after the original leaching, and at the time the *Rhododendron* cuttings were well rooted, was pH 6.76, showing practically no tendency toward reversion to the acid form. The results with *Rhododendron* are of particular interest,

since cuttings of this plant would be expected to respond the same as *Azalea*. COVILLE (4, 5) has shown that *Rhododendron* seedlings will grow well only in an acid medium, regardless of the amount or type of humus material present. It is possible, of course, that even though *Rhododendron* cuttings were readily rooted in a leached peat moss, they would not continue to grow for an unlimited period. Furthermore, even though root growth were favorable in leached peat moss, shoot growth might not be correspondingly good over a period of several years.

Neutralization of peat moss by alkaline reagents or by leaching with tap water causes the material to change from a light brown to a very dark brown, almost black. This change takes place within a day or two after the reagents have been added, and darkening continues as time goes on. If excessive amounts of alkali are added, the change in color is immediately produced. Change in color no doubt accompanies a chemical reaction. According to ITANO'S work (11), it seems quite possible that adjustment of the peat moss to a neutral reaction will allow of active bacterial decomposing action. The change just described may have some bearing on the difference in rooting obtained in used neutral peat moss.

Ligustrum japonicum rooted exceptionally well in sand. No roots were produced in peat moss during the time of the experiment. When the cuttings were transferred from peat moss to sand, however, roots appeared in two weeks' time. Such a result brings up the question as to whether the failure of root protrusion was due to lack of root initiation, or whether the root initials formed but failed to grow out through the bark. Although no anatomical studies were made on this material, it is believed that this phase of the general problem of vegetative propagation is extremely important. It is not definitely known whether for some kinds of cuttings different conditions in the rooting medium are required for root initiation from those required for root growth. Experiments with *Coleus* and mint cuttings indicated that for these particular plants differences in rooting in different media were due solely to factors which influenced the rate of root growth.

The efficiency of a mixture of peat moss and sand appears to be due mainly to its relatively high moisture-retaining capacity, to the

presence of growth-promoting materials furnished by the peat moss, to efficient aeration, and in some cases (*Azalea*, for example) to its acid reaction. The part which sand plays in this mixture (aside from that of dilution) is not clear. Both peat moss and sand are well aerated, so that efficient aeration in the mixture cannot be due to sand alone.

It appears from the results with cuttings that root growth may not be confined to such narrow ranges of pH values as has been supposed. Complex relations furnished by solid materials and many kinds of ions in rooting media must be considered as having a pronounced modifying influence on the extent to which the hydrogen-ion will exert its particular influence on root growth. SMITH's work on *Coleus* has an interesting bearing on this phase of the subject.

SMITH (20) found that root formation in *Coleus* cuttings attains a maximum in liquid media at pH 7.0, whereas in coconut fiber excellent rooting was obtained at a much more acid value (pH 4.5–4.7). This fact appears to be of particular importance, since the pH value of coconut fiber is comparable with that of a mixture of sand and peat moss. The writer found that in acid peat moss (pH 3.6) and in neutral sand *Coleus* cuttings rooted equally well. In the mixture of sand and peat moss (pH 4.1–4.4) root growth was invariably better than in either peat moss or in sand. The pH value of this mixture, as well as that of coconut fiber, was found by SMITH to be the acid limit (that is, pH 4.5) for *Coleus* cuttings placed in liquid media.

In view of these results with *Coleus*, it does not appear that data obtained for cuttings rooted in liquid media can always be used to explain the limiting factors for root production in solid media. SMITH's statement that, other conditions being equal, a neutral reaction of the medium was best suited for root production in *Coleus* cuttings, cannot be regarded as a very safe guide, for it is these "other conditions" which are often of the greatest importance. Differences in time of root protrusion at different pH values found by SMITH were not noted by the writer for *Coleus* cuttings placed in peat moss and sand. Observations by the writer included an examination of cuttings in pots of media and in glass tubes. In the latter case, cuttings of *Coleus* were placed next to the glass and fully

exposed to view. Roots appeared from cuttings in peat moss, in neutral peat moss, and in sand at the same time, at least during the same day, yet very marked differences in rate of root growth were observed thereafter in the different media.

Whether peat moss was furnished in its natural acid state, neutralized, or mixed with sand, a more rapid rate of root growth occurred in a medium containing peat moss than in one containing only sand. This brings up the question of the nutrient conditions which are furnished by peat moss. REID (18) pointed out that for tomato cuttings a low nitrogen value in the medium was more favorable for root growth than a high one. Such a result, however, was dependent upon the availability of carbohydrates in the cutting. Whether this relation holds true for other kinds of cuttings has not as yet been demonstrated. In order to gain some idea of the nutrient value of peat moss, tomato and buckwheat seedlings were grown in soil, natural peat moss, neutral peat moss, acid sand, and in neutral sand. Both of these species were able to grow to maturity in peat moss. The growth of tomato in natural peat moss was noticeably retarded during the first few weeks, but later the plant continued in what appeared to be a normal rate of growth, and set fruit. The tomato placed in neutral peat moss was at all times in a more vigorous state of growth than any of the others, including that in soil. Buckwheat did not show such marked variation in the different media, all plants setting fruit and attaining about the same vegetative growth. While this particular set of experiments cannot furnish any quantitative information, it shows that the nutrient value of peat moss is sufficiently high to be an important factor in determining the type of response in this medium.

BOTTOMLEY'S experiments (1, 2) with "bacterized peat" showed that the products of bacterial action of peat would stimulate growth of certain plants, especially that of *Lemna minor*. Raw, unbacterized peat would not do this. In connection with this work it is interesting to note the results which ITANO obtained with a Michigan acid peat. The normal alkaline permanganate soluble nitrogen was increased from 5.9 to 71.5 after adjusting the medium to pH 7.0 and adding accessory food materials containing vitamin B. Whereas BOTTOMLEY obtained his "growth-promoting substances" (auximones) from bacterial action on peat, ITANO added "growth-pro-

moting substances" to peat in order to promote bacterial decomposition.

The variation in chemical and physical properties of different types of peat, as shown by DACHNOWSKI (8), makes a comparison of growth responses in various types of peat or peat moss media a somewhat doubtful procedure. No doubt the failure of some workers to repeat BOTTOMLEY'S experiments has been due to the fact that the type of peat was different from that used by BOTTOMLEY. DACHNOWSKI makes a definite distinction between "peat" and "peat moss." The type used by the writer is designated "granulated peat moss." This is a standard commercial product which comes from bogs in Germany. It corresponds to the type which DACHNOWSKI classifies as a poorly disintegrated bog moss type (Section C, No. 7, p. 19). Over a period of three years this peat moss has proved to be of uniform texture and of the same acid reaction.

While the use of peat moss as a rooting medium for cuttings is by no means new, its use previous to 1925 for such a purpose in this country was noticeably limited. During the last two years, however, peat moss has been used rather extensively, not only as a medium in which to root cuttings, but also as a means of improving soil conditions. According to frequent reports in *Gartenwelt*, peat moss (torfmull) has been in more general use in Germany than in this country, but no attempt has been made to furnish detailed experimental data on comparative rooting responses of cuttings in peat moss and in sand.

Summary

1. According to their rooting response in peat moss and in sand, 96 varieties of cuttings (including 46 genera) have been classified into three groups. Cuttings which rooted readily in peat moss but poorly in sand are placed in group I; those which rooted readily in sand but poorly in peat moss are placed in group II; cuttings which rooted readily in either peat moss or in sand are placed in group III.

2. The fact that cuttings in all three groups rooted readily in a mixture composed of equal proportions of peat moss and sand (with the exception of five varieties in group II) indicates that this mixture is superior to sand as a general medium in which to root cuttings. Although the pH value of the medium was an important factor in determining the type of rooting response of some varieties

of cuttings, it was not the single limiting factor. The critical acid value, at which injury to the cuttings listed in group II occurred, was found to lie between pH 3.6 and 4.1. For the same varieties of cuttings callus formation was inhibited at pH values more acid than pH 4.1.

3. Whether peat moss was furnished in its natural acid state, neutralized, or mixed with sand, a more rapid rate of root growth occurred in a medium containing peat moss than in one containing only sand. Good rooting occurred for most varieties of cuttings over an acid range of pH 4.5–7.0.

4. Uniformity of rooting response of *Azalea amoena* cuttings in peat moss is attributed to the efficient moisture-retaining capacity of this medium. An increased moisture content of sand, as furnished by auto-irrigation, showed that in many cases, but especially under conditions of high light intensity, a more favorable rooting response was obtained.

5. For *Coleus* cuttings the conditions in the medium influenced the rate of root growth rather than the time of root protrusion. Cuttings of *Ligustrum japonicum* which failed to root during two months in peat moss, rooted in two weeks when transferred to sand, indicating that root initiation had probably taken place, but that unfavorable conditions provided by peat moss prevented root protrusion.

6. The efficient buffer capacity of peat moss was found to be due principally to the solid material, and not to the solutes in an extract. Methods for preparing samples and for making pH determinations are described in detail. Extracts of peat moss were found to give higher pH values than heavy suspensions.

Experiments here reported were carried out in the laboratories of the Boyce Thompson Institute for Plant Research. The writer is greatly indebted to members of the staff of the Institute and to Professors R. A. HARPER and S. F. TRELEASE of Columbia University for helpful suggestions and criticisms given during the progress of the work.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.
YONKERS, N.Y.

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EXPLANATION OF PLATES V–VII

PLATE V

FIG. 1.—*Azalea amoena* cuttings rooted in different media: *A*, peat moss medium (pH 3.6); *B*, bank sand medium (pH 5.8–6.2); *C*, Bay sand (Long Island, N. Y.) medium (pH 7.0+).

FIG. 2.—*Ligustrum vulgare* cuttings rooted in different media: *A*, peat moss medium (pH 3.6); *B*, bank sand medium (pH 5.8–6.2); *C*, Bay sand (Long Island, N. Y.) medium (pH 7.0+).

PLATE VI

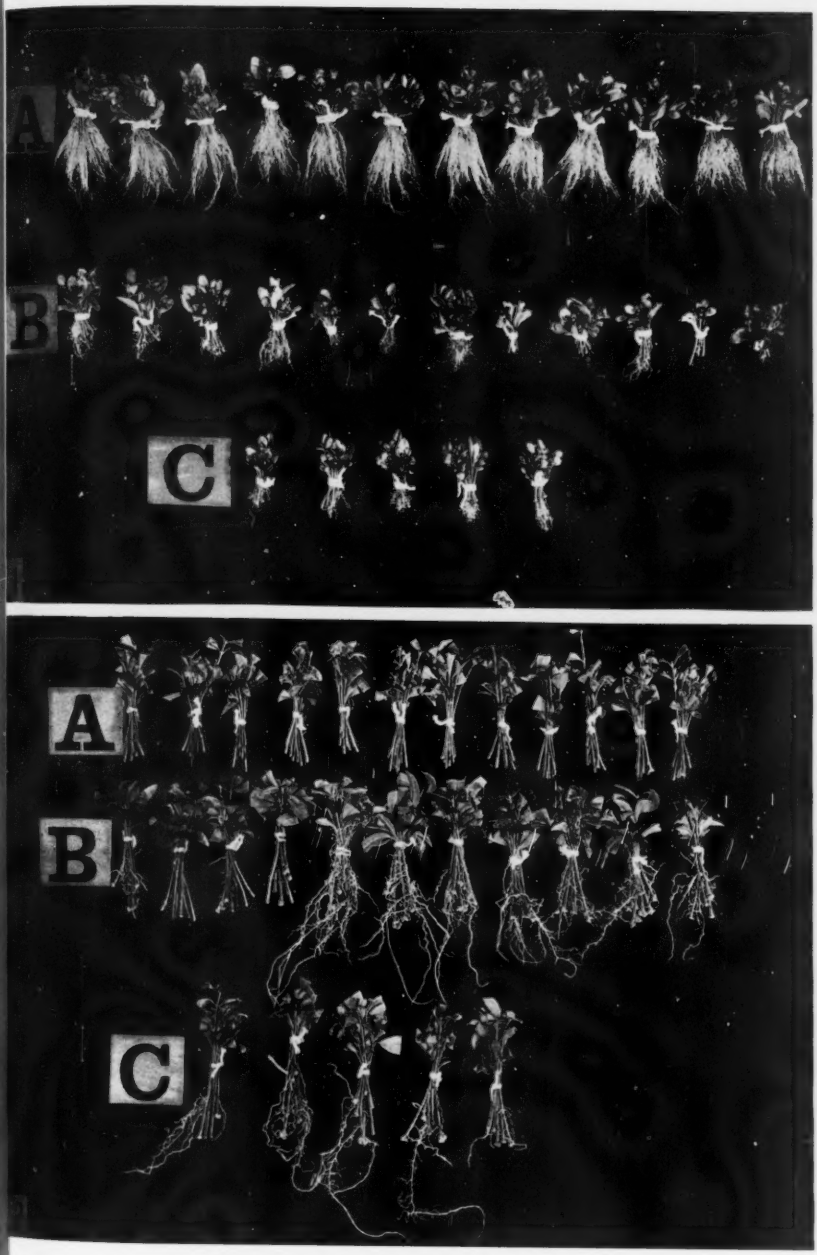
FIG. 3.—Cuttings of *Prunus glandulosa* rooted in different media: *A*, sand medium (pH 7.0+); *B*, mixture of peat moss and sand (pH 4.1–4.4); *C*, peat moss medium (pH 3.6); *D*, neutral peat moss medium (pH 7.0–7.3); *E*, mixture neutral peat moss and sand (pH 7.0–7.2).

FIG. 4.—Cuttings of *Mentha piperita* rooted in different media: *A*, sand medium (pH 7.0+); *B*, peat moss medium (pH 3.6); *C*, neutral peat moss medium (pH 7.0–7.3); *D*, neutral peat moss (pH 7.0–7.3) for ten days, then transferred to peat moss (pH 3.6); *E*, peat moss (pH 3.6) for ten days, then transferred to neutral peat moss (pH 7.0–7.3).

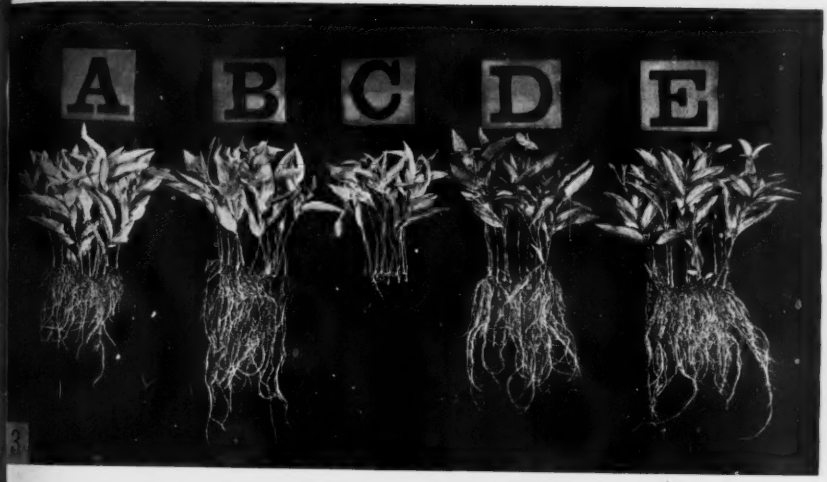
PLATE VII

FIG. 5.—Cuttings of *Ligustrum vulgare* (top row) and *Ligustrum ibota* var. *regelianum* (bottom row) rooted in different media; effect of auto-irrigation also shown: *A*, peat moss medium not irrigated; *B*, peat moss medium auto-irrigated; *C*, neutral peat moss medium not irrigated; *D*, neutral peat moss medium auto-irrigated; *E*, sand medium not irrigated; *F*, sand medium auto-irrigated; *G*, sand medium not irrigated (this particular lot of cuttings was shaded daily until 1:00 P.M.).

FIG. 6.—Cuttings of *Prunus tomentosa* rooted in sand: *A*, auto-irrigated; *B*, check (not irrigated).



HITCHCOCK on ROOTING RESPONSE



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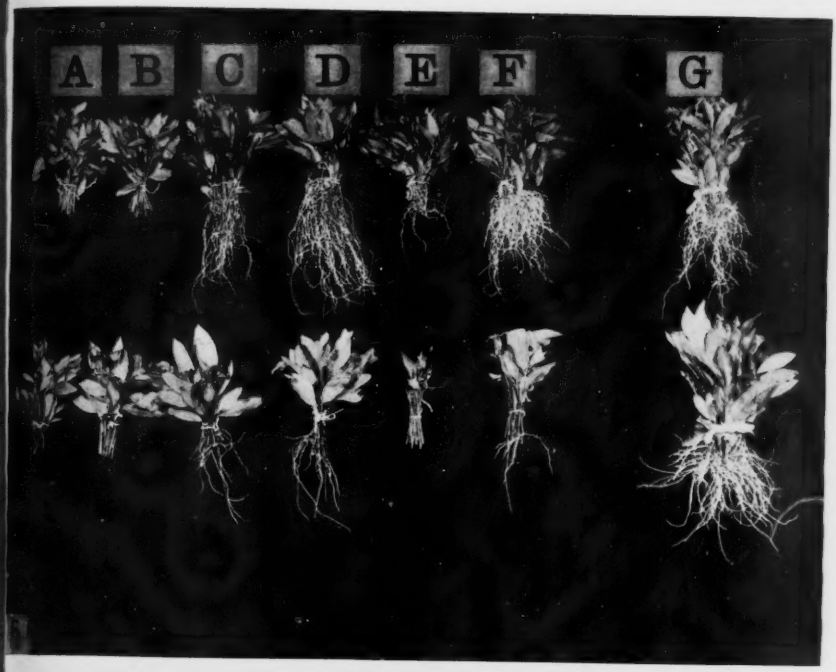

HITCHCOCK on ROOTING RESPONSE

MORPHOLOGY OF SASSAFRAS IN RELATION TO PHYLOGENY OF ANGIOSPERMS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 383

GEORGIA V. COY

(WITH PLATES VIII, IX)

Introduction

The question of the origin of vascular plants has stimulated both investigation and speculation, particularly in regard to the obscure beginnings of the Angiosperms. Botanists continue to investigate and speculate and they continue to be baffled, but slowly their lines of reasoning converge.

Paleobotany, once merely a study of outward form, in recent years has included more and more the investigation of internal structure. It has become most productive, and promises to be increasingly helpful in clearing up relationships. There is evidence of this, for example, in the publications on the Rhynie fossils of the Scottish Old Red Sandstones of the Devonian. These fossils have caused some shifting of the lower stretches of the phylogenetic lines, and seem to indicate at least the direction in which may lie their ultimate connections. ARBER (1) brought together the available material on Devonian fossil plants and divided them into two floras. The earlier and simpler of these, the Psilophyton type, were land plants with rootless rhizomes having dichotomously branching, but leafless, thalloid-like stems with terminal sporangia of several wall layers. These he considered progenitors of the plants of the Upper Devonian levels that made up his "Archaeopteris flora," and which were true though simple vascular plants, the most primitive Pteridophytes known. As a result of his studies, he derives all higher plants from the Algae by three independent lines, the Sphenopsida, the Lycopsida, and the Pteridopsida; with the Psilotales forming a fourth but much later one.

KIDSTON and LANG (21) have also published, among others, the description of a Devonian plant (*Hornea*) that indicates Bryophyte

affinities by its sphagnum-like sporangium with a columella and cuticularized spores in tetrads. It has a tuberous rhizome comparable with the embryonic protocorm of some of the Lycopodiales. Although the gametophytes are unknown, making comparison with Bryophytes correspondingly difficult, the investigators consider that the Rhynie fossils, so obviously related to the three great lower plant assemblages, have materially lessened the gaps between them.

SCOTT (25) calls attention to evidence from the Rhyniaceae indicating that the sporangium may be but a modified branch. KIDSTON and LANG (22) in fact refer to sporangia as representing remnants of Rhynaceous branch systems. This may cause some revision of ideas on sporophyll and sporangiophore, because the Devonian plants with their distinct terminal sporangia are regarded as leafless, not because of reduction, but because of their extremely primitive character.

Ferns and seed plant line

The position of the ferns in the evolutionary sequence is somewhat variously regarded. With respect to the group itself there is a tendency, even in the face of the modern trend toward the polyphyletic idea (as for instance, in ARBER'S scheme) to consider them as representing development from a single line. In SCOTT'S opinion, the characteristics long regarded as favorable to this view, such as the prevalence of an alternation of generations that stresses the sporophyte phase, the uniform mode of development of reproductive organs, and a similarity of vascular and stomatal histology, have been augmented by the discovery of their possible ancestors among the Devonian plants. He thinks, moreover, that the ferns are not in the direct line of Spermatophyte descent, because, rather paradoxically, the Cycadofilicales are too nearly like the Filicales to have been their descendants. Their great similarity would necessitate, in that case, an affinity too close to permit of the important differences between them, such as heterospory and the seed habit. The evidence of history seems to support this view, in that there are Middle Devonian Cycadofilicales or Gymnosperm stems (*Paleopitys*); but there are no records of ferns in the Early Devonian,

where they would naturally be expected if they had given rise to the higher forms.

Among those who regard the ferns as the progenitors of the seed plants is BERRY (9), who considers that Pteridophytes must have developed heterospory early in their history, because of the fact that the Cycadofilicales were undoubtedly derived from ferns of at least Pre-Devonian age. COULTER and CHAMBERLAIN (12), while granting that a possible Lycopod descent is worthy of consideration, hold the Gymnosperm derivation through *Gnetum* as untenable, and through Coniferales as unlikely, but look upon the early heterosporous Pteridophytes, with their generalized conditions of stem anatomy and reproduction, as a favorable point of departure for the Angiosperm line.

On the other hand JEFFREY (19) believes that Monocotyledons and Dicotyledons have not come directly from any group of vascular Cryptogams, either living or extinct, since the only organ in which they show the characteristic features of the organs of Cryptogams is the root, a fact without significance since all roots are alike in that respect.

However opinions may differ as to the true place of the ferns, or in regard to the relationship of the Cycadofilicales to other plants lower in the scale, these primitive seed plants, admittedly a large and heterogeneous group, are thought to stand at the point of divergence of all the higher Spermatophyte lines. Emerging from this Cycadofilicales plexus, the two main evolutionary series, those of the Cycadophyte and the Coniferophyte succession, carry on seed plant development with affinities varying according to the interpreters.

Seed plants

The Gymnosperms as a group have a long history and rather clearly indicated relationships, but this does not apply to the Gnetales, for which no fossil records have been found; a fact that, coupled with some resemblances to the Angiosperms, has made them the subject of much discussion. The characters which they display in common with the higher forms include the posesssion of true vessels in the secondary wood, companion cells in the phloem, a similar mode of sporogenesis and of fertilization, the reduction of

the gametophyte, some features of the embryo sac, embryo development, and, according to some, the floral morphology.

On the other hand, the researches of LANG and THOMPSON have shown them to be Gymnosperms. THOMPSON (29) finds that *Ephedra* shows conifer traits in the arrangement of the primary bundles, the double leaf trace, the disposition and structure of the pits on the tracheids, the bars of Sanio, tertiary spirals, lignified rays, wood parenchyma, and the endarch vascular bundles of the leaf. Their origin he considered to be near the base of the conifer line.

In view of the lack of fossil evidence, ARBER and PARKIN (2) suggested three possibilities as to the past history of the Gnetales: first, that they are aberrant survivals of a great and complex group dominant in the Tertiary vegetation; second, that they are a new group; third, that while in existence in the Tertiary they have always been a little varied and subsidiary element of the flora. The result of their study is the conclusion that they are nearest the Angiosperms of any of the several Gymnosperm lines, and that they are examples of parallel development from a common ancestor. This ancestor they would place among the Bennettitales, with the idea that Gnetalean floral organs have been derived from them by the reduction common to the evolution of an inflorescence. They cite *Populus glauca*, an Indian species described by HAINES, as an illustration of a similar process among Angiosperms, and one that furnishes an almost complete homology with *Welwitschia*.

JEFFREY (19) definitely opposed this idea, saying that the flower of *Welwitschia* offers no basis for comparison with a Bennettitalian cone, and that the similarity of the latter to an Angiosperm flower is entirely the superficial one of arrangement. He contrasts what he calls the epidermis-lined pollen chamber of *Ephedra* with the lysigenous one of lower Gymnosperms, and the Cycad fertilization by motile sperms with the siphonogamy of the Gnetales. In short, he holds that neither the Gnetales nor the Angiosperms are related to the Cycadophytes, but that there is no reasonable doubt of a Gymnosperm ancestry. This view is in accord with THOMPSON'S conclusions that the morphology of *Ephedra* affords no support for the idea of a Bennettitalian connection, and with the deductions

of BAILEY (4), BAILEY and TUPPER (7), and Miss BLISS (10) from their studies of vascular elements.

BERRY attributes to the Gnetales an ancient and collateral relationship with the other Coniferophytes, perhaps as far back as the Cordaitales region or the Cycadofilicales, and on the other hand through *Gnetum* with an unknown ancestor that may have produced the Angiosperms as well. In this connection it is interesting to recall the queries of CHAMBERLAIN (11) as to the possibilities of a lost herbaceous Gymnosperm flora that might have given rise to the herbaceous Angiosperms, that in turn produced the woody types of the Cretaceous; a theory that would obviate the otherwise evident need of deriving them from woody forms already known, the Cycadophytes or the Coniferales. There are some generalizations in WIELAND'S (31) paper also that may have point here. They are to the effect that there is a very small record of upland vegetation of past times, and that the flora of uplands and polar regions included the majority of plastic forms. The plants that were at once extinguished and preserved by subsidence made up the aplastic coastal floras.

WIELAND traces the Conifer line back through the Jurassic and Permian, the periods during which their inflorescences acquired the compact unisexual cone form, and connects them with the dominating Cordaitales of the Carboniferous. To Angiosperm alliances he finds no clue except the, to him, obvious one of the much varied Mesozoic Bennettitales. The slender forked stems of *Williamsoniella*, with their narrow pinnately veined leaves and small bisporangiate flowers, in WIELAND'S opinion "demonstrate the presence of a great alliance related to the primitive magnolias and abundantly represented in the leafy records of the Mesozoic." The period of dominance of this great Cycadeoidean assemblage, extending as it does from the Paleozoic to the Cretaceous, was particularly fruitful in the evolution of the higher forms; and WIELAND points out that some East Andean representatives of the group were upland and scrub types, while one of them (*Dictyozamites*) even had netted veined leaves.

SCOTT (25) subscribes to this idea of Angiosperm derivation, and admits the possibility, in view of the indicated relationships between

Bennettitales and Gnetales (ARBER and PARKIN 2), of a future reference of the whole Gymnosperm division to that ancestral stock. He regards the Gymnosperms as monophyletic, an opinion which SEWARD has also recently expressed. A considerable discussion of the relative merits of a Lycopod and Cordaitalean connection for the Araucarieae, which he thinks are primitive, brings him to the conclusion that the points in common with the Cordaitales carry much the greater weight. He judges that the Araucarian cone scales are too complex for comparison with Lycopod sporophylls, and that there is little likeness between the ligules of the two groups. The decision is reached, therefore, that on the whole there is evidence sufficient to establish for the Araucarian tribe, and with them the other Gymnosperms, a real affinity for the Cordaitales, not however through the Cordaiteae. For the Cordaitales in turn, despite the dissimilar habit and advanced character of their fructifications, there is a clear connection with the Cycadofilicales on the basis of seed structure and stem anatomy.

SINNOTT (26), using the leaf trace and foliar bundle characters as criteria, has erected a phylogenetic series that is especially interesting when compared with the alliances just described. It may be summarized as follows: The primitive foliar bundle, single, monarch and mesarch, characterizing the *Lycopsida* is evidence of their primitive nature and uniformity. Its presence in the base of the leaf trace of *Osmunda* and some of the Ophioglossales indicates their early separation from the other ferns. The typical Paleozoic Filicales had a diarch, mesarch trace which has reached the endarch condition in the modern ferns by the disappearance of the centripetal wood. The derivation has been, apparently, through the ancient Botryopterideae. Furthermore, from forms related to the latter, but which had already acquired the seed habit, have descended the Spermatophytes along two independent lines. The first of these, represented by *Calamopitys*, *Lyginodendron*, and *Heterangium*, was characterized by fernlike habit, peculiar seeds, double leaf trace, and the association of the protoxylem with the centrifugal wood to produce ultimately the endarch stele. This line ended blindly. The other succession includes all the remaining Gymnosperms and probably the Angiosperms. Its early members showed less fernlike habit

(*Medullosa*), a higher type of seeds more like those of Cycads, a double leaf trace in most of the series, and a tendency for the protoxylem to become associated with the centripetal wood and to develop the exarch stele. From this exarch series developed two principal groups, the Coniferophytes, represented by Cordaitales, and the Cycadophytes, with the Bennettitales and Cycadales as members.

The salient points of this brief review of opinion may be summarized as follows. The Angiosperms are admitted to exhibit Gnetalean resemblances to the extent of warranting the supposition for them of a common ancestor. The Gnetales are accorded a place among the Gymnosperms, and these in turn are derived from the primitive seed plants through the Cordaitales. The Cycadales and Bennettitales represent a varied assemblage tracing its origin along another line to the Cycadofilicales. Contradiction is encountered in the efforts of some authorities to associate the Angiosperms and possibly the Gnetales with the early Cycadophyte series. From the Cycadofilicales the succession passes downward, in the opinion of some through the Pteridophytes; in that of others parallel with them, to the primitive vascular plants that exhibit traits of Algae, Bryophytes, and Pteridophytes and are of so simple and generalized a character as to qualify as progenitors for all the higher forms. With increasing knowledge, however, there is discernible a growing tendency to suspend judgment. We find WIELAND (31) observing that "The lengthening of the fossil record shows nearly universal parallelism instead of comparatively recent development of dichotomous stocks. Slowly converging lines replace the paleontologic tree" of which the branchings are rapidly being pushed back on anatomical grounds; and SCOTT (25) warns that "A more tentative and diffident tone seems to be demanded in discussing phylogenetic problems."

Primitive Angiosperms

Regarding the past history of this division of plants, two things may be said: that it is suprisingly short as plant records go, and inexplicably abrupt in its initiation. Records of Angiosperms of undoubted character and certain age have been assigned only as far back as the upper levels of the Lower Cretaceous. To the previous

evidence of leaf impressions Miss STOPES (28) has now added the surer testimony of structure to prove that these earliest known Angiosperms were not in any sense proangiosperms or primitive types. Both the Monocotyledons and the Dicotyledons appeared among the remains, and BERRY (8) calls attention to the fact that they were already an important element in the vegetation. They were practically all Archichlamydeae, but it is noteworthy that the epigynous *Eucalyptus* had appeared among them. By the close of the Upper Cretaceous most of the principal families were evolved, with the exception only of the highest; the orchids and the composites and Angiosperms had gone far toward attaining their present dominance. In Tertiary time the flowering plants were much modernized, and the Miocene floras differed from the present mainly in greater prevalence of the arborescent types. Referring to this "crowning achievement of plant evolution," BERRY says "one is almost tempted to see design in the world-wide radiation of flowering plants during the Upper Cretaceous times immediately preceding the age of mammals."

Herbaceous Angiosperms

The further development of plant forms since the Miocene has resulted in the gradual usurpation of dominance by the herbaceous Angiosperms. This is the modern phase of which such groups as the Cruciferae, Labiatae, and Compositae are examples, and of which the history is apparently almost entirely post-glacial. SINNOTT (27) writes of the importance to man of this advance, in that it is closely correlated with the evolution of birds, insects, and mammals. The herbs as the subjects of primitive agriculture doubtless first enticed man from the forest, and have furnished the means by which he has wrought out his civilization.

The forces to which SINNOTT ascribed these changes resulted from disturbances of the uniformity of geological climates, the appearance of seasonal variation in temperature and moisture; that is to say, refrigeration and aridity. Among plants, then, the survivors were the very hardy woody types or the individuals so adapted as to hold over in the form of resistant seeds or hibernating underground stems. Organizing the evidence of floristics, general and

endemic, of distribution, fossil records, and plant anatomy, SINNOTT concludes that herbs are of recent and northern origin, a theory also expressed by WIELAND (32).

In comparison, there is no doubt of the antiquity of the woody forms and some of the older stocks, such as the *Equisetum* and *Lycopod* giants of the Carboniferous, are still represented by their degenerate descendants. In the whole curve of plant evolution, however, they stood at a point well up from the bottom. As to the character of the herbaceous plants that might have produced them, we are perhaps granted more than a glimpse in the discovery of the Devonian fossils; but these simple protostelic plants are separated by long periods of elaboration and modification from their dynamic progeny, the herbaceous vegetation of today.

The principal mechanical accompaniment of the evolution of herbs has been the breaking up of the siphonostele into a ring of bundles separated by parenchymatous tissue. To account for this, the most prominent hypothesis is that of the development of the rays; that is, the accumulation of storage tissue adjacent to the leaf traces as the result of greatly increased assimilative power, and their final disposition along the sides of the bundles as the vascular cylinder became progressively narrowed. When these bands of parenchyma extend from pith to cortex the stele consists of a ring of disconnected bundles, the typical herbaceous condition.

The possibility of using the pith ray systems in dicotyledonous woody stems, as indices of phylogenetic arrangement, has led to a great amount of investigation and to somewhat contradictory results. Among the various workers were THOMPSON (30), BAILEY (5), GROOM (15), Miss HOLDEN (17, 18), BAILEY and SINNOTT (6) who gave a very comprehensive report embracing fossil as well as living forms, Miss FLINT (13), and Miss LANGDON (23). Considerable interest attaches to the last named paper, in view of the conflicting results of the others. Miss LANGDON worked from a new approach, her object being to discover the possible modifying effects of age, location on the tree, and conditions of growth on the ray systems. The effects of the first two she regarded as negligible, but the ray systems were found to be so altered by differences in vigor of growth that she was led to consider them unreliable as indices of phylogeny.

For the polystele characteristics of Monocotyledons and of members of the Araliaceae and Umbelliferae a dicotyledonous origin is generally conceded. The great number of traces from the large parallel-veined leaves is commonly held accountable for the scattered arrangement of the bundles in the stem. But Miss SARGENT (24), writing in 1908, ascribed the characteristic differences in detail to the presence or absence of cambium. She used as illustrations certain Dicotyledons that have lost their cambium and developed a scattered arrangement and occasionally amphivasal structure. Miss SARGENT observes that cambium, while absent only in some Dicotyledons, is not really a functioning tissue in any of the Monocotyledons, although they still give evidence in their ontogeny and in some mature stem vestiges of its having been a factor in their phylogeny.

Another employment of dynamic tissues as an explanation of the disintegration of the siphonostele is made by LAND in his course in Spermatophyte morphology. He most interestingly demonstrates the performance of the pericycle in elaboration of the polystele from the unbroken dicotyledonous cylinder. The pericycle is the direct descendant of the primary meristem of the root and stem tips, and retains its potential activity, exercising it, in the presence of functional stelar cambium, only in the production of lateral members in roots.

The tendency to thickening of the pericycle cells of the stem causes, in many forms, the formation of patches of thick-walled cells capping the primary bundles. From this stage, LAND'S material shows a series in the progressive encroachment of the pericycle bands upon the bundles, severing the connections of the interfascicular cambiums, finally forming a completely enveloping sheath and reducing the siphonostele to a ring of disconnected bundles, the first step in its disintegration. The process is closely correlated with the evolution of amphivasal structure. In the rhizome of *Acorus*, for example, can be found an excellent intermediate stage. The pith bundles are amphivasal and are inclosed in a ring of bundles retaining the siphonostele arrangement but exhibiting varying degrees of amphivasal development, while in the cortex the scattered traces are uniformly collateral.

With the passing of the cambium, to which LAND refers as a transitory tissue appearing late in geological history, the pericycle again becomes meristematic, and lays down a broad zone of cells among which appear strands of secondary desmogen that in turn give rise to the bundles of secondary growth. This is well illustrated in the stems of *Aloe pleuridens*.

The present evolutionary tendencies of plants are picturesquely summarized by LAND: "The polystele and the rhizome lie at the end of every phylum. Toward the attainment of this condition among Monocotyledons, for example, wheat is well on the way and the banana has arrived."

Present classification of Angiosperms

As expressive of the attitude of many botanists toward the ENGLER scheme of classification, the following words of BERRY (9) are quoted: "While very imperfect and founded to too great a degree on floral morphology, the classification proposed by ENGLER and derived largely from EICHLER'S work is the most satisfactory and has the additional advantage of having been elaborated in a general systematic treatise"; but there is much opposition to the arrangement, especially as it applies to the primitive members of the Angiosperm group.

ARBER and PARKIN (2) bring together considerable evidence of reduction in the floral structure of Amentiferae which to them indicates derivation from higher forms. They hold with GOEBEL that the bisporangiate flower is primitive, and that plants with unisexual flowers represent a higher stage of development. They constructed a hypothetical primitive flower which they called the proanthostrobilus, and which was based on the bisporangiate Bennettitales cone described by WIELAND. From forms possessing flowers more or less like this they would derive the Angiosperms, with the Magnolias as the nearest living representatives. Thus they believe the Ranales to be the primitive members of this line. JEFFREY (19) sees little anatomical basis for this Bennettitales connection, and considers the question beyond solution without an acquaintance with the Angiosperms of the Jurassic or even earlier Mesozoic epochs.

COULTER and CHAMBERLAIN (12) express the opinion that the

primitive character of the bisporangiate flower is not a necessary assumption; that the majority of simple flowers should be regarded as primitive; and that this idea is in accord with the evidence of morphology and of history, as well as with the doctrine of evolution.

An extensive discussion of the subject, especially as it applies to the Fagaceae, may be found in HOAR'S (16) paper, with a review of the opinions of the investigators. A unique classification of the Centrospermae and allied families, based on F. MALLIGSON'S "serodiagnostischen" researches, is graphically shown by KARSTEN (20); and it is interesting to note that, while the Amentiferae hold together fairly well, they are placed upon a branch originating in the region of the Berberidaceae.

In favor of the Amentiferae as primitive forms there is historical evidence of their great antiquity, the generalized condition of the archesporium, the prevalence of wind pollination, and the many admittedly primitive characters of *Casuarina*. Perhaps the most obvious deduction from a glimpse into the literature of the subject is that there is great need for continued morphological work among this heterogeneous assemblage.

With the hope that a detailed study of the organogeny of the flower in some of the earliest known of living Angiosperms might help in the solution of the problem, an investigation of *Sassafras variifolium*, *Betula alba*, *Alnus glutinosa*, and *Corylus americana* was begun. Only the results of the study of *Sassafras* will be presented at this time.

Morphology of Sassafras variifolium

HISTORY AND DESCRIPTION

Sassafras, as a subject for investigation, is interesting from several standpoints. It is a monotypic genus of great antiquity, once widespread but now much restricted in range; it is a member of the great and varied order of Ranales, thought by many botanists to include the primitive living Angiosperms; and it has reached that stage of development represented by a distinctly unstable dioecism.

The fossil history of *Sassafras* in America is as long as that of any Angiosperm. It appears in the geological records in the Patapsco, the uppermost of the three Potomac formations of the Lower

Cretaceous (BERRY 8, 33). There it is associated with *Populus* and other early forms. COULTER and CHAMBERLAIN (12) mention *Sassafras* fourth in their list of Lower Cretaceous Angiosperms, giving chronological precedence to *Populus*, *Liriodendron*, and *Magnolia*.

According to BRITTON and BROWN (34), there is one species of *Sassafras* typical of eastern North America and another of Asia. The plant is described by them as follows: "A tree sometimes one hundred and twenty-five feet high, the trunk seven feet in maximum diameter, bark rough in irregular ridges, aromatic, the young shoots yellowish green, the twigs and leaves mucilaginous . . . . , leaves oval, or entire becoming mitten-shaped, or three-lobed to about the middle." In regard to this last statement, BERRY (33) calls attention to the fact that some trees have the leaves regularly four- or five-lobed, while occasionally there are specimens with six lobes. In the Chicago region, where *Sassafras* figures mostly in the dune vegetation, it attains only a moderate height and would be ranked as a small tree or shrub.

Materials and methods

Most of the material used grew in the vicinity of the southern shore of Lake Michigan, but some was obtained from northeastern Missouri. Dr. E. QUISUMBING, who had collected a variety of forms for work in connection with his studies on the stony layer in Gymnosperm seeds, very generously furnished *Sassafras* material already imbedded in paraffin, and this important assistance is gratefully acknowledged.

The collections extended over a period of ten months, from late October to late July, but the unavoidable length of some of the intervals has left some gaps in the sequence which it is hoped can be filled in the future. Very satisfactory results were obtained by the use of hot corrosive sublimate acetic in 70 per cent alcohol as a killing and fixing agent. Tissues that proved refractory when fixed with chromoacetic acid cut easily when prepared by this method. Haidenhain's iron haematoxylin and gold orange were the stains used for the most part, although some good preparations were made with alcoholic safranin and aqueous crystal violet.

MORPHOLOGY OF FLOWER

The flower is described in Gray's manual as "dioecious or nearly so, with a six-parted calyx; the sterile kind with nine stamens inserted on the base of the calyx in three rows, the three inner with a pair of stalked glands at the base of each, anthers four-celled; fertile flowers with six short rudiments of stamens and an ovoid ovary. Drupe ovoid, supported on a club-shaped and rather fleshy reddish pedicel."

The ovary of the Lauraceae, to which *Sassafras* belongs, is one-celled; and in ENGLER's syllabus (**36**) the number of carpels is recorded as "3 (oder 1?)." On this question MIRANDE (**37**), after a study of several of the genera, stated that all show evidence of trimerous pistil formation, but that two of the carpels abort early and only one is continued into a style and stigma. The preparations of *Sassafras* studied for this investigation showed three fibrovascular bundles in the transverse sections of the upper part of the ovary, and a blind opening near the base of the style. The former would seem to indicate the trimerous condition in that part of the structure, and the latter may correspond to the "ovarian canal" of MIRANDE's description. In the absence of intermediate stages in the growth of the pistil, however, that phase of the floral development must be left for further investigation.

In the youngest available ovulate flowers, collected April 7, the pistil appears as a conical elevation at the bottom of a cuplike depression that bears on its rim the sepals and the rudiments of stamens (fig. 1). In the very young stage shown in fig. 2 there is little differentiation, except that of the epidermis and the large gland cells, so conspicuous a feature of the young tissues. In some of the flowers of this date were found slightly more advanced pistils showing the beginning of elongation (figs. 1, 3), but without any evidence of sporogenous tissue or carpel structure.

The next step encountered showed considerable advance, the ovary with style and stigma developed and the ovule already partially anatropous (fig. 4). A stout funiculus attaches the ovule well up on the ovule wall, and above this point a very narrow channel passes outward from the ovary cavity to the surface, doubtless indicating the line of closure of the carpel. The appearance at this time is

very similar to that of a young carpel of *Ranunculus*. Only one integument was discernible at this stage. In one case two large megaspore mother cells were found (fig. 5), one of them with the nucleus in synapsis, the other one with its nucleus in the resting stage. This ovule is particularly interesting because it occurred in a bisporangiate flower.

The material collected on May 5 showed the mature, 8-nucleate, typically angiospermous embryo sac. It is long and rather narrow, with deeply placed antipodals which are inconspicuous and evanescent, but with egg apparatus and fusion nuclei well developed. The pistil has at this time attained a length of about 4 mm., and the ovule itself, nearly a fourth of this, fills the ovary cavity. It is now completely anatropous, suspended by its overarching funiculus. The micropyle is formed by the beaklike development of the inner integument, that is compressed below into a narrow row of cells, which, however, is easily traceable on either side nearly to the base of the nucellus. The outer integument is more massive, but at this time shorter than the other. The nucellus is pointed at the micropylar end and projects well up into the opening. Periclinal divisions have produced five or six layers of cells between the embryo sac wall and the surface. The peripheral layers of the nucellar tissues are compact, but those surrounding the embryo sac are composed of large cells, thin-walled and delicate, that make the outlines of the embryo sac itself difficult to distinguish.

After fertilization, endosperm development is rapid, and a thin layer of cytoplasm with many free nuclei lines the embryo sac by the time of the first division of the egg. In the formation of the first wall of the proembryo, *Sassafras* seems to agree with *Leitneria* as described by Miss PFEIFFER (**38**). Its plane apparently is not fixed, since 2-celled embryos were found, showing that it may be either vertical or horizontal.

When wall formation begins in the endosperm those cells nearest the embryo are of moderate size; but the greater part of this tissue is composed of extremely large, thin-walled cells which are conspicuous at as late a stage as that shown in fig. 19.

There is further agreement with *Leitneria* in the character of the cells that produce the flat-topped proembryo and the massive

embryo with its short, wide suspensor. The development during May is shown by figs. 11–16. Fig. 17 represents the condition reached early in June: a large mass of cells still without definite indication of the cotyledons. The cells of this embryo had a peculiar appearance due to extreme vacuolization, a detail of which is given in fig. 18.

The cells of the nucellus form a loose thin-walled perisperm about the embryo sac; and the ovary cavity, which now measures about 6 mm. in length, appears when cut open to be filled with a glistening transparent jelly. The peculiarly shaped embryo, with its widespread cotyledons, occupies but a portion of this space, since it measures only 1.3 mm. in width and scarcely 0.5 mm. in length. The cotyledons develop winglike lobes (figs. 19, 20) that extend beyond the hypocotyl and completely inclose it. The body of the embryo is still massive. In the most advanced stage examined, one taken from a ripening fruit, the stem tip had retained its broad top and was capped by the curving tips of the first leaves (fig. 20). The cotyledons at this time filled the whole interior of the ovule, measuring nearly 7 mm. in length, and had absorbed all the available food supply, their cells being packed with starch. The perisperm was reduced to a thin enveloping skin. Well developed spiral vessels were evident in the axis and in the fibrovascular bundles of the cotyledons.

Stony and transfusion layers

The behavior of the tissues surrounding the nucellus is interesting. Quisumbing (39) has briefly described the development of the stony layer in *Sassafras* as a palisade growth of the epidermal lining of the ovary. The elongation of these cells about the micropylar region of the ovule may be seen in fig. 8. By the time the embryo has reached the condition indicated in fig. 19, these cells have become very prominent, long, thick-walled, and pitted; but in the sections cut at right angles to the faces of the cotyledons, and therefore across the prominent ridge that is formed around the upper portion of the stony coat, the differentiation ends abruptly about a third of the way down from the micropyle. The lower cells of this layer are at this time oblong in section and show no thickening of the lateral walls. In the ovule itself considerable transfusion tissue shows at this period, developing apparently in the outer integument.

About the apex of the nucellus this is several layers thick, and composed of large cells, but it is reduced below to one or two layers of narrowly oblong cells.

When the fruits containing embryos of the largest size figured were cut, the whole inner layer of the ovary wall was hard, although perceptibly less resistant in the region of the pedicel. Its development, then, seems not to be of uniform progression, but the later stages of modification must take place rather rapidly with the ripening of the seed.

Staminate flower

The staminate flower presents an interesting topography on account of the 4-chambered stamens with their valves, and the conspicuous paired glands at the bases of the inner row of filaments. In the center of the normal flower there is a rudimentary pistil very similar to that represented in fig. 2, although its cells appear to be even nearer active development.

The staminate glands are reniform in longitudinal section in the plane of their greater width. They have a rich vascular supply, spiral vessels spreading fanwise from the stalk and frequently ending in direct contact with the large gland cells. The latter, characteristic of all the tissues, are particularly noticeable here because of their number. They first show as somewhat enlarged cells with homogeneous cytoplasm and a central nucleus. Then vacuolization begins and increases until the cell content is crowded into a thin peripheral layer with a shrunken and flattened nucleus. Eventually the protoplasm disappears and the cell, several times its original size, becomes filled with the characteristic fluid. In sections from older flowers, especially in the region of the thickened pedicel, canals occur that apparently have been formed by the breakdown of contiguous cells of this description.

Microsporangium

In stamens of flowers gathered in November there were found microspore mother cells in prophase and others in anaphase of the heterotypic division, while many anthers contained fully formed microspores. The tapetum in all these stages is very conspicuous. It consists of large, deeply staining, often binucleate cells that begin to disintegrate before the microspores are formed, so that the young

pollen grains lie in the midst of a plasmodium with many flattened nuclei. Remnants of this plasmodium are still evident in the mature sporangia of flowers collected just before anthesis in the spring. The microspores give early evidence of abundant nourishment, and when mature are packed full of large starch grains (fig. 24). The exine is beset with short straight spines and the spores are shed in the binucleate stage. Flowers brought in May 10, 1923, were shedding pollen, but the season was nearly a month later than the previous one.

The opening of the anthers, which in *Sassafras* is by the rather spectacular arrangement of four uplifted valves, is accomplished by the development of a peculiar endothecium. The cells below the slightly rounded ones of the epidermis become elongated at right angles to the axis of the anther, and then are heavily thickened (fig. 24). A deep groove marks the dehiscence line along the sides and bottom of this area of heavy cells, but it does not appear at the top where the cells are especially conspicuous for their size.

Bisporangiate flower

A number of bisporangiate flowers were found, in every case with fertile stamens but with the pistils in various stages of development. Some of these were obviously merely cases of delayed abortion; but one, from which fig. 5 was made, had developed up to the megaspore mother cell stage with every sign of continuing. This specimen was from a collection made in November, and the appearance of its pistil is in marked contrast with that of the one shown in fig. 1, belonging to a supposedly normal ovulate flower, collected April 7 the following spring. This latter material was scanty, however, and it is possible that it may not have represented normal development of the pistil of that date. It will be interesting to discover whether or not that is the case, and also whether or not the bisporangiate flowers develop seeds.

Discussion

Inasmuch as *Sassafras* is the single survivor of an ancient race, of which much of the history is known, and is placed taxonomically in an order classed as genetic and primitive because of the generalized condition of many of its members, any primitive or distinctive characters in its morphology are worthy of note.

While the evidence of a possible reduction in the pistil and the anatropous character of the ovule indicate the accuracy of its classification as near the apex of the Ranales group, the formation of more than one archesporial cell in the nucellus, with indications that this tissue may be even more extensive, may be regarded as a primitive feature. Aligned with this also is the relatively massive nucellus. The common occurrence of the dioecious condition in the floral organization implies a history long enough to have permitted its evolution from the monoecious type; but its instability indicates a degree of development below that of many Angiosperms of more recent origin. In this respect, *Sassafras* falls within the number described by COULTER (**35**) as follows: "Many of the dioecious Angiosperms, however, seem rather recently to have been derived from ancestors which have two sexes represented in the same flower (or at least on the same plant). In these the dioecious condition seems not to have been firmly established; a regular sex chromosome mechanism has not yet been perfected," and is therefore much more subject to fluctuation under environmental influence than the long established unisexual forms.

Leaves of the lobed type like those of *Sassafras* doubtless represent modifications of an originally simple leaf, and this again signifies that much time has elapsed in the process of development. This genus, as already mentioned, is among the oldest of the Angiosperms known, but the earliest fossil records show that the Lower Cretaceous species of *Sassafras* had bilobed and trilobed leaves. With this feature carried so far, the possible character of the flower becomes an interesting subject for speculation. There might be pictured at least so primitive a condition as a bisporangiate flower, with three separate carpels on a common stalk, each bearing a single orthotropous ovule. From this it is not difficult to visualize the gradual abortion of the two carpels, and the development downward of the remaining one in such a way as to cause the suspension of the ovule from the upper part of the ovary wall and the ultimate assumption of the anatropous position.

Another point of interest from the standpoint of survival value is the evidence throughout the plant of high assimilative power and effective nutrition. Cross-sections of growing twigs disclose a pith laden with starch granules. The vascular supply to the floral organs

is conspicuous in all of the sections; the secretory organs are strikingly well developed; the microsporangia have a prominent tapetum and are later supplied with a plasmodium; the microspores are crowded with starch grains; the cotyledons are large and well supplied with food; and the seed is protected by a hard coat that appears to be remarkably resistant for the amount of stony tissue developed.

Summary

1. The one-celled ovary of *Sassafras* is normally borne in an ovulate flower in which there are 6 rudimentary stamens. The ovule is solitary, anatropous, and may form more than one archesporial cell. The nucellus is massive and has two integuments.

2. The embryo sac is of the typical 8-nucleate type, but the antipodals are small and evanescent. In the endosperm many free nuclei are formed before wall formation begins. The cells are small near the embryo but very large in the middle of the sac. The perisperm is conspicuous until a late stage.

3. The first division of the fertilized egg occurs when the free nuclear stage in the endosperm is nearly completed, and may be either longitudinal or transverse.

4. The embryo is massive, with short suspensor and peculiarly auricled cotyledons. It eventually fills the ovary cavity. The seed is without endosperm.

5. A stony layer is developed by the elongation and thickening of the innermost layer of the ovary wall. Transfusion tissue forms in the outer integument, noticeably in the micropylar region.

6. The microspores are formed in most of the sporangia by November. A plasmodium of tapetal origin is conspicuous. The microspores are binucleate at time of shedding.

7. Bisporangiate flowers are not uncommon, and show ovulate development apparently much in advance of the normal fertile flower.

8. While *Sassafras* is one of the earliest known Angiosperms, its Mesozoic records do not differ much from the corresponding features in the living genus which indicates a much more ancient, unrecorded ancestry.

The writer desires to express sincere appreciation of the constant guidance of Professor C. J. CHAMBERLAIN, under whose direction

the work was done; and of the inspiration and encouragement of the other members of the botanical staff of the University of Chicago.

STATE TEACHERS COLLEGE
SAN DIEGO, CALIF.

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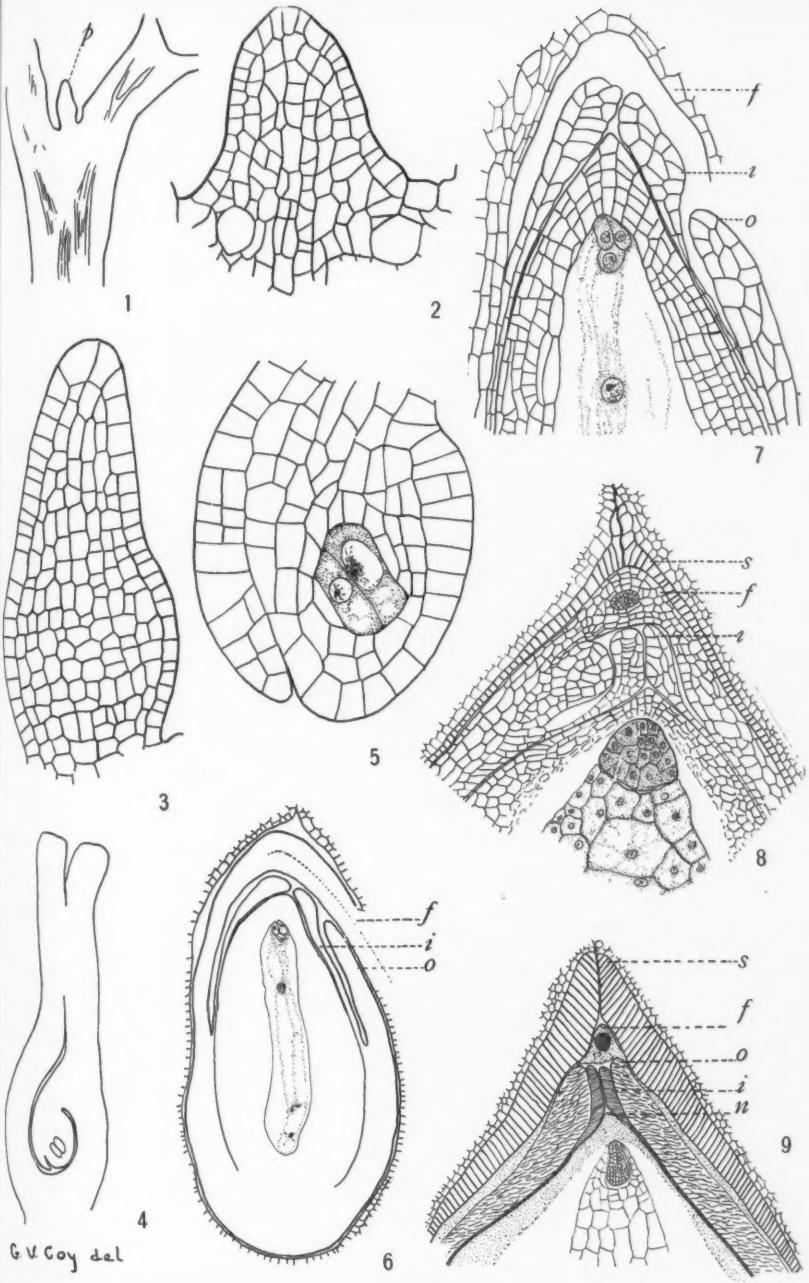
EXPLANATION OF PLATES VIII, IX

All drawings were made with the aid of the Abbé camera lucida. The abbreviations are as follows: *a*, antipodals; *f*, funiculus; *i*, inner integument; *l*, lobe of auricled cotyledon; *n*, nucellus; *o*, outer integument; *p*, pistil; *s*, stony layer.



COY on SASSAFRAS

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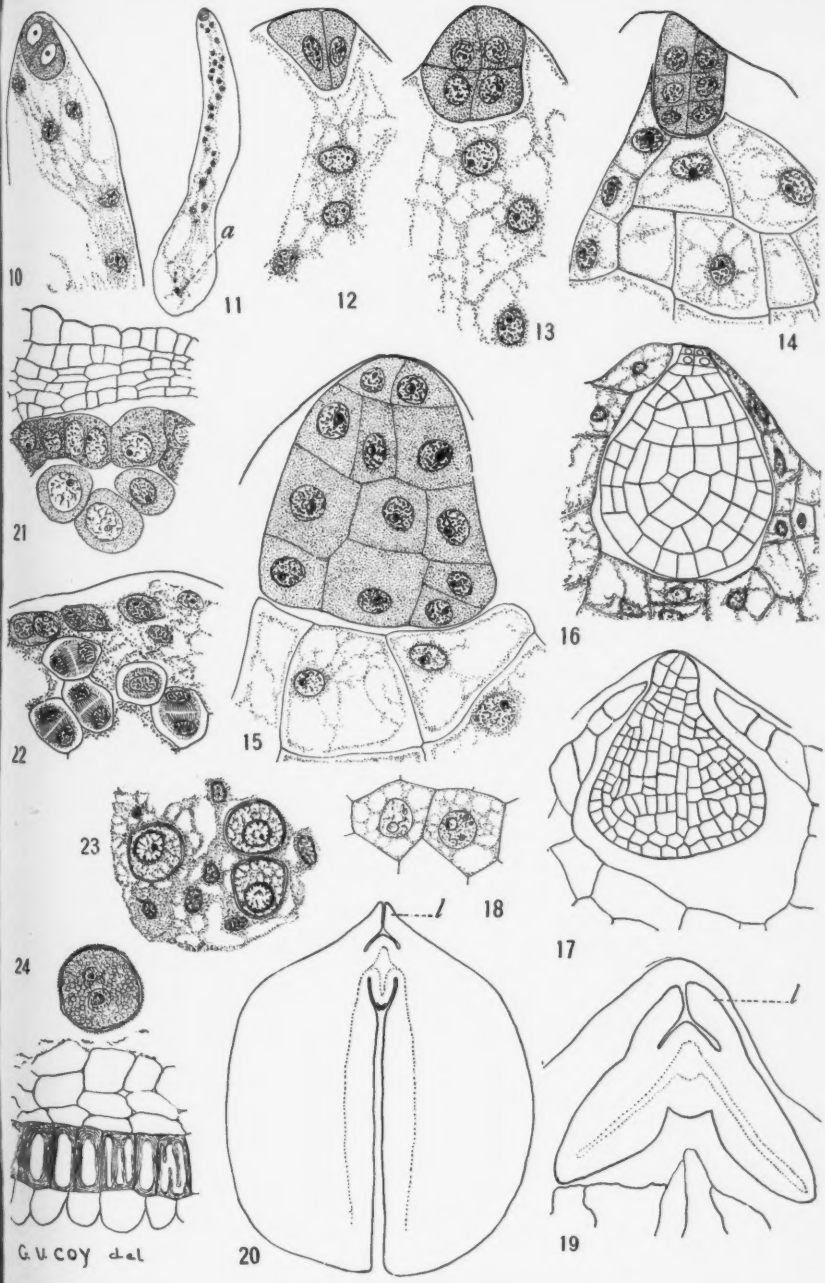

COY on SASSAFRAS

PLATE VIII

FIG. 1.—Longitudinal section of young ovulate flower showing pistil; ×25.

FIG. 2.—Enlargement of pistil somewhat younger than that in fig. 1, two gland cells visible on either side near base; ×210.

FIG. 3.—Enlargement of pistil shown in fig. 1; ×210.

FIG. 4.—Diagram of longitudinal section of pistil from bisporangiate flower of November 26, showing ovule and archesporial cells; ×80.

FIG. 5.—Enlargement of ovule from fig. 4, showing detail of archesporial cells; ×475.

FIG. 6.—Diagram of ovule with embryo sac before fertilization, showing position of funiculus and integuments, egg apparatus, fusion nucleus, and antipodals; ×80.

FIG. 7.—Enlargement of micropylar region of ovule shown in fig. 6; ×210.

FIG. 8.—Micropylar region of ovule with proembryo, portion of funiculus, and elongating cells of ovary wall that will form the stony layer; ×80.

FIG. 9.—Diagram of same region when embryo has reached stage shown in fig. 17, showing elongation of cells of stony layer and transfusion tissue in outer integument; ×50.

PLATE IX

FIG. 10.—First division of fertilized egg by horizontal wall; free nuclei in endosperm; ×350.

FIG. 11.—Topography of embryo sac at this stage; ×50.

FIG. 12.—First division of egg by longitudinal wall; ×475.

FIG. 13.—Second division of proembryo; ×475.

FIG. 14.—Proembryo showing further division and endosperm with walls formed; ×475.

FIG. 15.—Beginning of massive development of proembryo; ×475.

FIG. 16.—Embryo of many cells in globular mass, with short suspensor; ×350.

FIG. 17.—Embryo ten days older than that in fig. 16; ×210.

FIG. 18.—Detail of vacuolated cells of embryo shown in fig. 17; ×650.

FIG. 19.—Embryo showing well developed, auricled cotyledons; ×210.

FIG. 20.—Embryo extending full length of ovule and showing very large cotyledons and very small first leaves; ×10.

FIG. 21.—Portion of microsporangium showing wall cells, tapetum, and microspore mother cells; ×475.

FIG. 22.—Portion of microsporangium with spore mother cells completing heterotypic division; tapetal cells partially broken down and forming plasmodium; ×475.

FIG. 23.—Young uninucleate microspores surrounded by plasmodium; ×475.

FIG. 24.—Portion of microsporangium showing endothecium, inner wall layers; and microspore with tube and generative nuclei, and numerous starch grains; ×475.

YELLOWS OR LITTLE-LEAF OF WALNUT TREES[1]

A. R. C. Haas, L. D. Batchelor, and E. E. Thomas

(with five figures)

Yellows or little-leaf of the walnut is characterized by the growth of small, yellowish green foliage, which frequently serves to distinguish the disease at a considerable distance. The twig growth

Fig. 1.—Terminal twig growth of walnut affected with yellows

usually has extremely short internodes, with the compound leaves closely clustered together; and oftentimes the actual size of the leaflets is reduced to one-tenth or less of the normal size. The dorsal surface of the petiole of the compound leaf is curved convexly. The leaflets are commonly curved in a similar manner, and also tend to

[1] Paper no. 188, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

close along the midrib on the dorsal surface. This is well illustrated in fig. 1, which shows typical diseased terminal growth. The leaflets may have a yellowish green color, resembling that of chlorosis, or may show various degrees of mottling (fig. 2).

FIG. 2.—Walnut leaflets moderately affected with yellows, showing mottling

Marginal burning of the foliage is not necessarily typical of this disease, but may be present as a result of excessive salt content of the soil solution or other toxic conditions.

The root system of the diseased trees is characterized by a brownish, unhealthy appearance and a lack of fibrous rootlets.

In the advanced stages of the disease there may be an actual

dying back of the terminal growth. In extreme cases the disease has been known to kill entire trees, regardless of their age. Young trees from one to ten years of age have been known to recover from yellows without having received any remedial treatment; conversely, trees which have maintained a normal growth for a period of ten to fifteen years have been known to develop the disease gradually, even to the point of ultimate destruction.

SMITH ET AL. (7) maintain that walnut yellows is associated with abnormally long dry seasons, or any other soil or irrigation conditions which may bring about a prolonged abnormal dryness of the subsoil. HODGSON (1) attributed winter injury of walnut trees to nematodes when it was characterized by foliage reduced in size and distinctly paler than normal in color.

Walnut yellows seems to manifest certain symptoms in common with pecan rosette (fig. 3), mottling of citrus, and the little-leaf of apricot and peach (fig. 4). ORTON and RAND (5) believe that pecan rosette is nonparasitic and is not transmissible. They observed the disease on many different types of soils and under various cultural conditions, but were unable by means of ash analyses to show any difference in composition between diseased and normal trees. They state, however, that the disease is caused directly or indirectly by some soil relationship which brings about a lack of balance in the plant-food materials or some unfavorable physical condition, although no definite evidence is offered to substantiate this statement.

RAND (6) views pecan rosette from a pathological standpoint, and gives a very extensive review of the literature. MCMURRAN (4) believes that a deficiency of humus, fertility, and moisture supply is a causal factor in producing pecan rosette. MCCLINTOCK (3) was unable to obtain transmission of peach rosette by means of soil, or of sap from diseased trees. Likewise the insect transfer of the disease was considered improbable; nevertheless this author concludes that peach rosette may be transmitted from one tree to another "by means of buds."

Walnut yellows is widely distributed in southern and central California. Practically every walnut-growing district in the state has areas affected with this disease, regardless of climatic conditions. The soil types on which the disease most frequently occurs are as

follows: Hanford fine sandy loam, Placentia loam, Ramona loam, and Yolo clay loam.

FIG. 3.—Terminal twig growth of pecan affected with rosette

The use of irrigation waters with various amounts and kinds of impurities does not bear any direct relationship to the occurrence of this disease. Diseased trees are found growing in groves which

receive all extremes of cultural and irrigation conditions. These conditions range from dry farming to over-irrigation, and from clean culture to growth in sod or (dooryard trees) in driveways. No one type of walnut rootstock or variety of walnut seems more susceptible

FIG. 4.—Peach twig affected with little-leaf, compared with twig bearing normal leaves.

to the disease than any other. Our observations lead us to believe that there is a direct relationship between various tree troubles such as walnut yellows, pecan rosette, citrus mottling, peach little-leaf, and apricot little-leaf.

A study has been made of the occurrence of nematode (*Heterodera radicicola*) upon the roots of healthy and diseased walnut trees.

Not only the roots of diseased but also those of healthy trees were frequently found to be infested with the nematode; conversely, the roots of diseased and of healthy trees were frequently found to be free from nematode attack. It is obvious, therefore, that the presence of nematode infestation cannot be related to the occurrence of the disease.

Some investigators attribute the occurrence of these diseases to a low nitrogen content in the soil. No study seems to have been made regarding the effect of soluble salts upon the prevalence of these diseases. Table I shows the results obtained for analyses of 1:5 water extracts of soil from adjacent healthy and diseased groves. These results are typical of many such comparisons. Only the anion content of the water extracts was determined. The carbonate, bicarbonate, chlorine, nitrate, and sulphate contents are given in terms of parts per million of dry soil. A comparison of the concentration of the anions in the water extract of the soils shows no important differences between the soils from healthy and those from diseased groves. Our data lead us to believe that these diseases are independent of the nitrogen content of the soil, although this is contrary to the conclusion drawn by several previous investigators.

Previous workers have failed to consider the influence of base exchange upon the nutrition of the trees. Usually the base exchange phenomenon has not been considered when nitrogen has been applied to the soil. For example, when nitrate of soda is added to the soil, a considerable part of the sodium goes to replace the calcium and other bases, and as a consequence the soil solution is richer in sodium and nitrate but also is greatly enriched with calcium and other bases. As this type of fertilization is continued, a soil solution may become depleted by leaching of much of the soluble calcium and other constituents, and finally enriched in sodium. Also alkalinity may develop in consequence of these soil changes, and this may finally reduce the solubility of the calcium, iron, etc., in extreme cases. Deflocculation of the soil may also result from such base exchange. In the investigations herein reported these complicating factors of nitrate fertilization have been considered.

At the outset of the investigation healthy and diseased walnut leaves were collected from different localities, from such extreme

TABLE I

ANALYSES OF 1:5 WATER EXTRACTS OF SOIL FROM ADJACENT HEALTHY AND DISEASED WALNUT GROVES

DEPTH OF SAMPLE FROM SURFACE OF SOIL (FEET)	PARTS PER MILLION OF DRY SOIL											
	CO_3		HCO_3		Cl		NO_3		SO_4		Total solids	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
	Comparison I: adjacent orchards, Placentia loam											
1	0	0	92	107	27	27	4	6	Trace	Trace	210	210
2	0	0	107	107	18	18	Trace	Trace	Trace	Trace	160	220
3	0	0	122	122	27	35	Trace	Trace	Trace	Trace	185	170
4	0	0	122	107	18	18	Trace	Trace	Trace	Trace	165	130
5	0	0	107	107	35	18	Trace	Trace	Trace	Trace	165	200
6	0	0	107	107	35	27	0	0	Trace	Trace	100	185
7	0	0	107	107	27	35	0	0	Trace	Trace	160	270
8	0	0	183	198	27	27	0	0	Trace	Trace	165	265
9	0	0	183	183	27	27	0	0	Trace	Trace	165	275
10	0	0	198	213	27	27	0	0	Trace	Trace	200	265
11	0	0	167	183	18	27	0	0	Trace	Trace	200	275
12	0	0	183	183	18	18	0	0	Trace	Trace	185	120
	Comparison II: adjacent orchards, San Joaquin loam											
1	0	0	107	30	27	27	12	13	Trace	Trace	200	200
2	0	0	107	76	27	35	10	15	Trace	Trace	200	215
3	0	0	107	92	27	44	5	8	Trace	Trace	205	220
4	0	0	137	122	27	35	6	9	Trace	Trace	210	210
5	0	0	107	122	18	53	4	26	Trace	Trace	170	235
	Comparison III: adjacent orchards, Yolo clay loam											
1	0	0	107	107	44	44	53	53	Trace	30	445	460
2	0	0	92	92	53	35	13	40	Trace	Trace	195	255
3	0	0	107	92	71	44	7	12	Trace	Trace	185	225
4	0	0	76	92	27	35	13	6	20	Trace	200	215
5	0	0	107	92	35	27	Trace	7	20	Trace	185	235

conditions of tree growth as are illustrated in fig. 5. Analyses of the ash of such leaves gave very conflicting results, due to the fact that the leaves of the two types differed in age. This factor may account for the inconclusive results of many previous investigators in regard to the effect of the disease upon the composition of the

FIG. 5.—Extreme conditions of walnut tree growth in absence and presence of yellows.

leaves. After a great amount of preliminary work, it was finally decided to make first a study of the effect of age upon the composition of healthy and diseased walnut leaves.

The writers were fortunate in having available healthy and diseased trees growing upon the Experiment Station property. The past history of the occurrence of the disease upon the trees in question was well known, certain trees having been consistently normal, and other trees extremely diseased over a period of years. As new

shoots appeared on each class of trees, one could be quite certain of the ultimate nature of the resulting growth.

When the leaves on the two extreme types of trees were about one month old, tags were placed about the leaf stalks in order to identify leaves of the same age. At intervals of from five to seven weeks, samples of leaves of the same age were collected from healthy and diseased trees. These were dried at 70° C. and the composition of the ash was determined in the usual manner. The results are tabulated in table II. A study of the data of the trees on the Rubidoux tract shows a very rapid increase in ash content at the outset of growth, and later a slow but progressive increase until maturity is reached, after which there may be a slight decrease. By July 1 the ash content of the dry matter of the leaves is about three-fourths of that at maturity.

With the increase in the age of the leaves there is a decrease in the percentage of inorganic phosphate in the ash. The percentage of magnesium, chlorine, and sulphate in the ash remains relatively constant throughout the growth of the leaves. A matter of particular interest is the relation between the percentages of sodium and potassium in the ash, which decrease with increasing age of the leaves; and that of calcium, which is greatly increased as the leaf approaches maturity. It is of interest to note in this connection that the inverse relationship between the sodium and potassium percentages and the calcium percentage in the ash is quite analogous to that which exists in the ash of citrus leaves, as shown by KELLEY and CUMMINS (2). The calcium of normal mature walnut leaves usually ranges between 17 and 29 per cent of the ash.

When comparisons are made between healthy and diseased leaves of the same age, it is found that the percentage of potassium in the ash of the former is consistently lower than in that of the latter. A similar comparison shows that the percentage of calcium is consistently higher in the ash of healthy leaves than in that of diseased leaves. In this regard the ash of diseased leaves has the same composition as that of less mature normal leaves. This is precisely the same relationship that KELLEY and CUMMINS found to exist in the ash of healthy and mottled citrus leaves.

With this information with regard to the effect of the age of the

TABLE II

COMPOSITION OF ASH OF DRY MATTER OF HEALTHY AND DISEASED WALNUT LEAVES IN RELATION TO AGE

	Trees from Rubidoux tract								Trees from Box Springs tract							
	Healthy							Diseased	Healthy		Diseased	Healthy		Diseased	Healthy	Diseased
Date of sampling	5/6	6/6	6/17	7/31	9/28	10/20	11/10	5/7	6/23	6/23	8/5	8/5	9/28	9/28	10/20	10/20	11/10	11/10
Ash (as percentage dry matter)	6.46	9.01	10.33	10.99	12.79	12.90	11.81	6.65	10.16	9.71	11.22	10.76	12.57	11.88	13.05	9.95	13.92	11.77
	Ash constituents (percentage of ash)																	
Na	6.45	5.96	5.53	4.59	3.51	2.41	3.46	5.91	3.03	4.84	1.82	2.26	1.27	2.36	0.53	2.34	1.10	0.65
K	26.16	24.63	20.30	16.60	13.15	12.00	13.08	20.42	12.58	17.43	7.17	7.68	5.36	7.02	5.83	7.60	4.52	5.59
Ca	13.07	16.40	18.54	22.59	24.33	25.53	24.72	3.20	24.46	17.07	26.02	22.57	27.19	21.78	28.39	25.62	29.02	27.31
Mg	5.07	4.31	4.59	4.41	4.64	4.60	4.77	6.45	6.50	7.08	6.74	7.99	7.34	7.51	6.96	9.59	6.85	9.02
Cl	0.88	0.74	0.79	0.81	0.85	0.91	1.10	1.20	1.64	3.18	1.20	2.78	0.90	2.92	0.94	3.49	1.11	3.52
SO_4	4.08	3.44	3.13	3.69	4.49	4.01	4.25	4.08	3.54	4.59	3.39	4.27	3.27	3.83	3.50	4.63	2.74	4.06
PO_4	15.93	11.28	8.47	6.06	5.95	6.08	5.96	13.72	6.38	8.93	4.48	7.30	5.19	6.00	4.45	5.79	4.08	3.59

leaf upon the composition of the ash, additional samples of leaves of known similar age were collected from healthy and diseased trees in several localities. The data reported in table III further justify the conclusion, drawn from table II, that the ash of diseased leaves is higher in potassium and lower in calcium than leaves from healthy trees.

A comparison of the ash constituents of similar portions of healthy and diseased walnut trees is presented in table IV. The analytical data from the various portions of the compound walnut

TABLE III

COMPOSITION OF ASH OF DRY MATTER OF HEALTHY AND DISEASED WALNUT LEAVES OF SAME AGE

	Comparison I Chino		Comparison II Arlington		Comparison III Arlington		Comparison IV Anaheim	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Ash (as percentage dry matter)	10.56	9.73	10.48	9.61	10.73	11.55	12.15	14.50
	Ash constituents (percentage of ash)							
Na	4.80	6.10	3.48	5.25	4.83	4.92	4.53	6.08
K	20.34	24.27	12.79	23.06	18.31	19.89	17.62	21.02
Ca	18.37	14.80	22.13	13.90	17.48	13.87	20.83	17.87
Mg	2.60	2.52	5.83	5.68	5.77	6.76	5.88	4.76
Cl	1.44	1.86	.....	.....	.....	.....	4.52	3.18
SO_4	4.30	3.57	2.63	4.65	3.14	3.50	2.39	3.10
PO_4	5.53	6.67	13.02	12.66	15.15	17.58	12.01	8.15

leaves are comparable with those obtained from the complete leaves. The inverse relationship between the percentages of calcium and potassium in the ash of healthy and diseased leaves is further confirmed. The same conclusion may also be drawn from the ash analyses of the husks and the matured twigs of the last growth cycle. The differences obtained, however, may not be significant. Nearly half of the ash of the walnut husk is potassium.

The ashes of mottled citrus leaves and of walnut leaves affected with yellows are related because their ashes contain a higher percentage of potassium and a lower percentage of calcium than that of normal leaves. This same relationship is shown by table V to

TABLE IV

COMPARISON OF ASH OF DRY MATTER OF SIMILAR PORTIONS OF HEALTHY AND DISEASED WALNUT TREES

	Leaves		Leaflets		Leafstalks		Complete compound leaf		Matured last cycle of twigs		Husks		Kernels	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Ash (as percentage dry matter)	14.39	11.04	11.89	10.18	19.40	14.66	12.71	11.53	5.94	7.71	23.53	22.51	1.74	1.83
Ash constituents (percentage of ash)														
Na	1.35	3.93	2.78	3.66	1.82	3.64	2.02	4.08	1.31	0.92	10.30	12.89	7.13	5.76
K	9.41	14.07	10.41	18.49	5.98	13.22	8.18	17.23	5.28	6.04	42.61*	42.82†	21.29	23.27
Ca	28.19	22.54	24.64	16.34	30.84	23.76	26.93	18.07	32.53	29.98	6.44	5.89	4.91	5.03
Mg	4.52	3.42	4.84	4.26	4.90	6.72	5.08	5.06	3.99	4.08	1.20	0.86	1.50	1.72
Cl	1.05	1.80	1.64	2.01	0.50	0.58	1.29	1.97	0.15	0.26	0.63	1.18	0.37	0.52
SO_4	3.66	4.57	1.56	2.37	7.03	6.40	2.36	3.61	0.90	1.90	2.00	3.11	.....	.....
PO_4	4.00	16.39	3.66	4.57	1.49	1.88	3.15	4.07	10.71	15.28	3.54	7.64	.....	.....

* Equals 10.03 per cent of dry matter.
† Equals 9.64 per cent of dry matter.

apply equally well to leaves from pecan trees affected with rosette, and grape leaves affected with mottling and little-leaf, when compared with normal leaves of the same age.

In consideration of the abnormally low calcium and high potassium percentage in the ash of diseased walnut leaves as compared with healthy leaves, it is of interest to determine the water solubility of the inorganic constituents of the dry matter at increasing ages of the leaves. Table VI shows the relatively constant values obtained for the ash of the water-soluble fraction of walnut leaves of

TABLE V

COMPOSITION OF ASH OF DRY MATTER OF HEALTHY AND DISEASED PECAN AND GRAPE LEAVES

	Pecan								Grape	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
	Ash (as percentage dry matter)									
	9.42	8.21	8.65	8.69	8.43	7.45	10.13	8.06	5.26	9.90
	Ash constituents (percentage of ash)									
Na.....	2.74	4.13	3.35	4.17	3.52	4.16	3.84	4.41	5.21	9.02
K.....	11.37	16.72	10.59	13.97	13.16	15.82	12.36	17.70	21.28	31.29
Ca.....	22.33	19.27	23.51	19.91	21.58	19.77	22.95	17.76	13.42	11.13
Mg.....	8.21	7.03	7.92	6.94	8.44	8.20	5.61	6.99	6.86	2.74
Cl.....	0.83	0.91	0.74	0.82	0.33	0.80	0.32	0.84	0.84	0.91
SO_4.....	2.77	3.16	3.12	3.03	2.88	3.48	3.74	3.44	3.56	3.51
PO_4.....	7.15	9.52	6.86	8.94	6.73	10.54	6.70	10.85	20.12	44.70

varying ages, both healthy and diseased. The ash of the water-insoluble fraction shows considerable increase, however, with an increase in the age of the leaves. The data further show that the percentage of calcium in the ash of the water-soluble fraction of the dry matter of healthy leaves makes consistent but extremely small increases with increasing age of the leaves. In the case of diseased leaves, however, the percentage of calcium in the water-soluble fraction of the dry matter shows increases of much greater magnitude. In the dry matter of mature healthy walnut leaves practically all of the calcium is water-insoluble. This is not due to precipitation of the calcium because of any development of alkalinity in drying

the leaves, as a water extract of the ground twice-dried leaves of healthy trees gave a pH value of 4.84, which is somewhat more acid than the juice of healthy leaves. The percentage of magnesium in the ash of the water-soluble fraction of the dry matter of the walnut leaves increases with increasing age of the leaves, while that of potassium shows a decrease.

Table VI indicates that diseased walnut leaves may have a lower percentage of calcium in their ash than healthy leaves, but that the percentage of water-soluble calcium may be greater in the diseased than in healthy leaves, and it suggests that some toxic agent in the diseased leaves prevents some of the water-soluble calcium from becoming water-insoluble.

It was of interest to express the juice of frozen mature healthy and diseased leaves of walnut and make a comparison with that of healthy orange leaves. Such a comparison is presented in table VII, which shows that the ash content of expressed juice of orange leaves is from three to four times as concentrated as that of walnut leaves. The percentage of sodium, potassium, and magnesium in the ash of walnut leaf juice is much greater than that of orange leaf juice. The striking comparison is that the percentage of calcium in the ash of the expressed juice of orange leaves is about fifteen times as great as that of walnut leaf juice.

If we calculate the total amounts of the ash constituents from the data given in table VII, we find that the total amount of potassium in the expressed juice of walnut as compared with that of orange leaves may not differ greatly; however, the total amount of calcium in the expressed juice of walnut as compared with orange leaves is extremely low. KELLEY and CUMMINS (2) found a marked decrease in the percentage of calcium in the ash of expressed juice of citrus leaves when mottled as compared with that of normal leaves. We have found only a small difference, however, between the composition of the ash of the expressed juice of healthy and diseased walnut leaves.

KELLEY and CUMMINS also found the expressed juice of mature normal citrus leaves to have a greater pH value and less total acidity than the expressed juice of mottled leaves. In these respects the results for the expressed juice of normal and mottled citrus leaves

TABLE VI

WATER-SOLUBLE AND INSOLUBLE INORGANIC CONSTITUENTS OF WALNUT LEAVES OF DIFFERENT AGES EXPRESSED AS PERCENTAGES OF DRY MATTER

	Date of sampling											
	5/7/25		6/23/25		8/5/25		9/28/25		10/20/25		11/10/25	
	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.
	Leaves from healthy trees growing on Ramona loam											
Ash	3.15	4.33	3.15	7.87	2.54	9.06	2.72	10.80	3.61	10.63	2.86	11.70
Ca	0.01	1.21	0.02	2.49	0.03	3.06	0.03	3.50	0.03	3.62	0.04	4.06
Mg	0.18	0.27	0.28	0.37	0.44	0.40	0.49	0.36	0.50	0.37	0.61	0.36
Na	0.19	0.07	0.10	0.08	0.11	0.09	0.10	0.10	0.14	0.00	0.08	0.07
K	1.25	0.13	1.26	0.08	0.76	0.05	0.71	0.04	0.70	0.04	0.63	0.04
P	0.16	0.14	0.10	0.10	0.09	0.10	0.11	0.09	0.12	0.06	0.12	0.10
	Leaves from diseased trees growing on Ramona loam											
Ash	.....	.....	3.77	6.34	3.20	8.52	3.65	9.93	3.62	8.73	3.86	10.40
Ca	.....	.....	0.02	1.53	0.03	2.29	0.15	2.56	0.14	2.40	1.58	3.14
Mg	.....	.....	0.30	0.39	0.50	0.34	0.57	0.30	0.63	0.32	0.75	0.31
Na	.....	.....	0.11	0.10	0.18	0.10	0.11	0.09	0.29	0.21	0.12	0.08
K	.....	.....	1.47	0.12	0.79	0.07	0.91	0.05	0.81	0.07	0.66	0.05
P	.....	.....	0.18	0.10	0.21	0.10	0.15	0.11	.....	.....	0.08	0.09

were analogous with those we have obtained for the juice of healthy and diseased walnut leaves, the data for which are set forth in table VIII.

TABLE VII

COMPOSITION OF EXPRESSED JUICE OF LEAVES FROM HEALTHY AND DISEASED WALNUT TREES COMPARED WITH JUICE OF NORMAL MATURE ORANGE LEAVES

	Walnut leaves		Orange leaves
	Healthy	Diseased	Healthy
	Ash (gm. per 100 cc. of juice)		
	1.3516	1.5948	5.3325
	Ash constituents (percentage of ash)		
Na	9.20	9.66	4.18
K	44.18	43.07	14.49
Ca	1.85	1.57	24.98
Mg	5.67	5.26	2.39
Cl	11.24	13.06	.....
SO_4	2.92	3.36	5.15
PO_4	6.88	10.49	2.09

TABLE VIII

RELATION OF pH AND TOTAL ACIDITY OF 5 CC. SAMPLES OF EXPRESSED JUICE OF LEAVES FROM HEALTHY AND DISEASED WALNUT TREES

Total acidity (cc. of N/10 NaOH)	pH	
	Healthy	Diseased
0	5.14	4.36
1.15	6.91	5.65
1.73	7.13	.....
2.30	7.46	6.49
2.88	7.68	.....
3.46	.....	7.29
4.04	8.13	7.51
5.19	.....	7.91
6.34	.....	8.18

Samples of sap from the trunks of healthy walnut trees were collected by boring a slightly inclined hole into the trunk and attaching a threaded block-tin pipe, attached to a Pyrex flask protected from dirt, light, evaporation, etc. The pH of some samples

was as high as 8.2, and changed to 5.2 on standing. The pH of sap forced by means of compressed air out of the ends of freshly cut walnut shoots showed a pH of 5.2. The buffer value of the sap is probably very small, and fluctuations in the CO_2 content of the sap possibly account for the great variations.

Table IX shows the analysis of the 365 cc. sample of sap obtained by natural flow. The PO_4 determination was made on a separate 34 cc. sample of sap from the same tree. The potassium, calcium, and phosphate content are considerable.

To supplement the data of table IX we have obtained branches from healthy and diseased trees, and roots from healthy walnut

TABLE IX

COMPOSITION OF SAP OF NATURAL FLOW OBTAINED BY TAPPING A HEALTHY WALNUT TREE

ASH FROM 365 CC. SAP	0.1686 GM. OR 462 PARTS PER MILLION	
	Ash constituents (percentage ash)	Parts per million in sap
Na	5.88	27
K	22.83	105
Ca	18.80	87
Mg	4.15	19
Cl	0.84	4
SO_4	1.46	7
PO_4	21.15	98
Total S as SO_4	.....	38

trees (*Juglans hindsii*). Adapting the fundamentals of the method in use in Dr. BENNETT'S laboratory at Berkeley, we have withdrawn the sap from the branches and roots by means of a battery of six Pyrex suction flasks and the use of air suction.

Table X gives the results of the analyses of the sap obtained by suction. It is seen that a very close agreement exists in the concentration of the potassium from branches of healthy and diseased trees. The outstanding fact is the large concentration of calcium in the sap of the diseased as compared with that of the healthy branches. This agrees with the results on water solubility of the calcium of the dry matter of diseased walnut leaves as shown in table VI. Possibly the larger concentration of calcium in the

branches of the diseased trees is due to its accumulation as a result of the inability of the diseased leaves to utilize it in as great amount as normal leaves.

TABLE X

COMPOSITION OF SAP OBTAINED BY SUCTION FROM HEALTHY AND DISEASED WALNUT TREES (PARTS PER MILLION)

	HEALTHY BRANCHES	HEALTHY BRANCHES	DISEASED BRANCHES	HEALTHY ROOTS	HEALTHY ROOTS
Total solids	8105	4040	5176	4134	2328
Ash	653	565	885	.....	.....
Na	48	.....	127	5	.....
K	113	.....	111	109	.....
Ca	131	126	258	87	.....
Mg	.....	73	31	.....	.....
Total P as PO_4	.....	.....	.....	136	16

Discussion

We have shown that yellows or little-leaf of walnut trees has much in common with mottle-leaf of citrus, rosette of pecan, etc. For a practical demonstration of this fact we have selected the area of soil where plots G and H adjoin on the Rubidoux tract. Plot G has been fertilized annually for the past 13 years at the rate of 486 pounds of nitrate of soda, 500 pounds of dried blood, and 1067 pounds of steamed bone meal per acre per year. During the 7-year period prior to this, the same fertilizer materials were applied but in smaller amounts. Contiguous to plot G is plot H, which has been fertilized annually for the same period at the rate of 972 pounds of nitrate of soda per acre per year. The citrus trees which are growing on the land where these two plots adjoin each other are at present badly affected with mottle-leaf. In the interspace where these two plots adjoin we planted in the spring of 1925 several normal trees each of walnut, pecan, peach, and apricot.

The walnut trees are now badly affected with yellows or little-leaf. The apical growth is badly rosetted, and the leaves are either green with mottling very conspicuous, or are pale yellowish green and folded. The pecan trees show extreme cases of rosetted leaves, and the peach trees show typical little-leaf, which can be distinguished at a considerable distance. The peach leaves are narrow

and long, and frequently are mottled and folded dorsally along the midrib. The apricot trees are typical of the condition of many trees that are found growing in commercial orchards. Many if not all of the leaves of a cycle may be considerably reduced in size, while those of an adjoining cycle may be much larger. It is not uncommon to find considerable mottling among the smaller leaves.

Regardless of what may be designated the cause of mottle-leaf of citrus, we have shown herein that various troubles of the other kinds of trees used in these tests may be brought about by the same environmental conditions. On the other hand, when trees of the same lot were planted in soil in which citrus is free from mottle-leaf, no troubles of the kind described occurred. This strongly indicates that these troubles are due to soil conditions and not to infection. Controlled cultures in sand and soil are being conducted by the senior author, so as to learn more specifically the nature of these "physiological" tree diseases.

As to what has taken place in the soil of plots G and H owing to the fertilization, one can only speculate as yet. The bases of the soil no doubt have been undergoing exchange with the bases (principally sodium) in the fertilizer applied, with the result that calcium, magnesium, and potassium have been set free into the soil solution and sodium has largely taken their places in the soil complex. The bases of the soil solution may be absorbed by the roots, or carried below the reach of the tree roots by excessive irrigation or by rains, or may partially remain bathing the roots. In this way the roots are gradually being given a very different environment from that originally present. In soil cultures, by a replacement of the bases in the soil by means of potassium nitrate solution, it has been possible to produce rosetted pecan leaves.

The sodium complex may hydrolyze, moreover, and alkalinity may develop. The solubility of the iron, calcium, phosphate, etc., may thereby be gradually reduced. In line with this idea, the senior author is now conducting experiments with soil in which alkalinity is being developed by mixing sodium carbonate, potassium carbonate, or sodium aluminate with the soil and then planting budded citrus trees. Orange trees have been caused to mottle badly while the control trees are healthy. In this case no removal of bases by

leaching has occurred, and the only conclusion to be drawn is that alkalinity has prevented the trees from a full utilization, possibly by effects within the tree itself, of the bases (largely calcium) most needed in the maturing of the leaves.

One other possibility suggests itself, and that is that there may possibly be sufficient absorption of toxic agents, in exceedingly small amounts, to cause these leaf diseases. This possibility cannot be dismissed lightly, for experiments under way suggest that leaves affected with such toxic agents may be prevented from obtaining sufficient calcium to enable them to mature, and consequently may develop a condition resembling these physiological diseases.

The writers are convinced that in the soil of plots G and H, and in other experiments where the diseases considered are prevalent, there is abundant nitrogen, and that therefore nitrogen starvation cannot be a factor.

Summary

1. The yellows or little-leaf of walnut trees and the rosette of pecan trees, as well as little-leaf of the peach and mottling of apricot and of citrus, have been found to be independent of the nitrogen requirements, the anion content of the water extract of the soil, climatic factors, cultural and irrigation practices, nematode infestations and soil types, but appears to be dependent upon the base relationship in the soil. These tree diseases are "physiological" and not due to infection.

2. The ash of affected leaves is lower in calcium and higher in potassium than that of healthy leaves of the same age.

3. The percentage of calcium in the ash of the water-soluble fraction of diseased leaves increases as maturity is approached, and finally becomes considerably greater than that of healthy leaves. Nearly all of the calcium of the dry matter of healthy walnut leaves is insoluble in water.

4. The sap drawn by suction from diseased walnut branches shows a greater concentration of calcium than that from healthy branches.

5. The ash content of walnut leaves increases very rapidly at the outset of growth, and then shows a slow but progressive increase until maturity is reached, after which there may be a slight decline.

By July 1 the ash content of the dry matter is about three-fourths that at maturity.

6. The expressed juice of orange leaves is from three to four times as concentrated as that of walnut leaves in ash content. The percentage of calcium in the ash of the expressed juice of orange leaves is about fifteen times as great as that of walnut leaves.

7. Practically no differences exist in the ash composition of the expressed juice of healthy and diseased walnut leaves.

8. The expressed juice of diseased walnut leaves has a lower pH value and a greater total acidity than that of healthy walnut leaves.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIF.

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CHEMISTRY OF GROWTH AS REPRESENTED BY CARBON/NITROGEN RATIO[1]

REGENERATION OF WILLOW CUTTINGS

PHYLLIS A. HICKS

(WITH EIGHT FIGURES)

Introduction

The power of regeneration is to some extent common to all living organisms, but the factors governing regenerative response are still insufficiently understood, and a general theory of regeneration is hardly possible at the present state of knowledge. Even regarding the simplest form of regeneration, namely, the production of a shoot from a dormant bud, there are conflicting views.

PFEFFER (20) believes that there are two forms of rest period, autonomic, or internally caused, and aitionomic, or externally caused. KLEBS (11), however, maintains that the cause is entirely external, while HOWARD (6) states: "The beginning of rest, particularly in woody plants, appears to be wholly due to inner causes, but before the complete state of rest is reached outer conditions may help to hasten the rest." In support of his statement, KLEBS brought hyacinth and celandine into a hotbed in the greenhouse, checked the resting period, and had two flowerings a year. KLEBS is supported by FLAMMARION (3), who grew seedlings from acorns under greenhouse conditions for fifteen years, by the end of which time the young trees became almost like evergreens, the new leaves appearing before the fall of the old ones.

If the rest period were caused entirely by external conditions becoming unfavorable to growth, it should be broken upon transfer of the plants to favorable conditions. HOWARD (6), however, has proved that many plants fail to grow when brought into a warm greenhouse, either in November or January. MOLISCH (16) used warm baths and radium, JOHANNSEN (8) and STUART (26) ether or chloroform, JESENKO (9) weak alcohol, MCCALLUM (18) ethyl bro-

[1] Part of thesis accepted for the degree of Ph.D. of the University of Wales.

mide, HARSHBERGER (5) ammonia, BOS (1) a galvanic current, LAKON (12) various nutrient solutions, and WEBER (28) mechanical injury. Even these many and varied expedients failed to stimulate growth in all cases, although they were effective in many at certain times of the year. Thus the theory of external causes is not adequate.

Internal conditions, such as sugar or starch accumulation, have been held responsible by the supporters of the autonomic hypothesis, but analyses have shown that the rest period begins or is broken quite independently of the amount of these substances present; and, indeed, the amount of these compounds is different every year. These facts lead us to suppose that neither external nor internal conditions alone are responsible for initiating or breaking the rest period, but that they interact.

As regards the physiology of the plant, the rest period is not a phase of entire inaction. As a result of his experiments, SIMON (24) states: "So far as some of the functions of growth and metabolism and also respiration are concerned, woody plants have no rest period, for even in winter under favorable conditions these activities reach a relatively high intensity." Similarly, FISHER (4) shows that the reserve materials in most trees undergo marked transformation during that period. Thus with the study of regeneration, in so far as it concerns the opening of buds only, we have not to deal with "awakened" metabolism, but merely with stimulated processes.

SMITH (25) suggests the possible importance of the C/N balance in regeneration, while SUMMERS (27) discusses the possibilities of this more fully, and, quoting the memoir of BUTLER, SMITH, and CURRY (2), concludes that the awakening of vegetation is synchronous with the translocation of nitrogenous reserves to the growing regions.

MÜLLER-THURGAU (17), working on potatoes, has shown that growth is preceded by sugar being made available as fuel for the greatly increased respiration, the sugar being formed by diastatic ferment of reserve starch or other carbohydrate. This is substantiated by FISHER'S statement that resting buds of trees cannot be made to grow until a certain minimum of insoluble starch has been changed "to easily respired and therefore energy yielding material."

JOHANNSEN and PFEFFER entirely disagree that the presence of sugar is a controlling factor, while other workers show that increased respiration is not alone responsible for regeneration. This again points to the value of a "food" balance as a possible controlling agent.

In considering regeneration of roots as well as shoots, other important factors demand consideration. The first of these is polarity. One of the greatest workers on this problem has been, without doubt, LOEB (13). As a result of a great number of experiments on *Bryophyllum*, he accounts for the tendency of all cuttings to form shoots at the apex and roots at the base in the following way. Specific hormones or formative materials are supposed to exist in the plant, each responsible for the growth of a particular type of structure. Thus the growth of roots at the lower end of the shoot is assumed to be due to flow of root-forming substances to the lower end of the cutting, and shoot-forming substances to the apex. Inversion of the shoot, however, will cause a reversed polarity after a time, so that the supposition that the shoot-forming substances were specifically lighter than those responsible for root production is inadequate. The objection that, on severing a long stem into several cuttings, the apex of one is practically the base of the next, was surmounted by assuming that the redistribution of the hormones may be modified by gravity, as, for example, through drainage of root-forming materials away from the anterior cut end of the posterior piece, rendering the growth of roots impossible, while the root-forming materials are unable to escape from the posterior cut end of the anterior portion. The presence of leaves upon the cutting affects root formation and geotropic curvature in amount, according to their basal or apical situation on the stem. How far this hypothesis of LOEB may be modified by the investigation of changes in the C/N ratio will be discussed later.

Investigation

This investigation upon the variation of the carbon and nitrogen relations during normal regeneration, together with the effects of increasing and decreasing the initial C/N ratio, was begun in February 1927.

Cuttings from the winter shoots of a variety of *Salix viminalis* from the Roath Park Botanical Gardens were suspended in Kilner jars containing a little distilled water, lined with blotting paper also moistened with distilled water. Two sets of normal cuttings were arranged, one set suspended in moist air only, and the other with the base of the cutting dipping into the water.

Previous workers had found that regeneration was stimulated by etiolation, a fact which they thought to be due to the reduction of carbon, and hence of the C/N ratio. Analysis, however, showed that the etiolated plants were full of starch.

Another attempt at discovering the effect of sugars upon regeneration had been made by placing a leafy shoot into sugar solution, transpiration thus supplying the pull. Unfortunately the sugar passed into the leaves, which then dropped off. In the present experiment sugars were drawn into the shoot by means of a suction pump, and the cut ends sealed with a rubber tube and glass cap. Prior to this the amount of carbon and nitrogen already in the stem had to be ascertained. The epidermal layer was removed and the underlying tissues, consisting of cortex, phloem, and cambium, were scraped off, dried to constant weight at 100°C., and the two elements estimated by the PREGL (21) micro-analytical methods described fully in part I.[2] Total elemental nitrogen and total elemental carbon were thus estimated. These tissues, afterward spoken of collectively as "bast" tissues, were chosen as representing the chief seat of regenerative activity. SMITH has shown that the adventitious roots in *Coleus* arise from a nest of highly meristematic cells in the cambium, the appearance of which seems to be followed by enzyme action, since sections show a gap suggestive of digestion in the area of the advancing root tip. KLEBS also uses these tissues to determine enzyme activity during regeneration, the wood being discarded as showing too slow an enzyme action. A very few estimations of the wood, however, were made in this study.

The bast tissues were found to be low in nitrogen, 1.623 per cent, and high in carbon, 34.48 per cent (C/N 21.2). This high carbon value is due chiefly to salicin, which is stored in the cortex of the

[2] HICKS, PHYLLIS A., The carbon/nitrogen ratio in the wheat plant. New Phytol. 27: April 1928.

willow. The salicin is normally split nightly into glucose and saligenin, but in autumn it tends to accumulate, owing to the reduced activity of the enzyme salicase. Thus glucose is the translocation product of salicin, a fact which will be of importance later.

The second series of cultures was then so arranged that the shoots possessed at least double the C/N ratio existing in the bast tissues of the normal shoots. The possible difference of availability of several sugars was allowed for, and glucose, saccharose, lactose, and levulose, together with the glucoside salicin, were used. The strength of the solution which had to be drawn up by the twig to produce the desired increase of carbon content was calculated as shown by the following example.

1. Relation of dry weight of bast to wet weight of twig:

Wet weight of twig..... =0.7263 gm.
Wet weight of bast in twig..... =0.1283 gm.
Dry weight of bast in twig..... =0.07 gm.

$$\text{Ratio: } \frac{\text{Dry weight bast}}{\text{Wet weight twig}} = \frac{0.07}{0.72} = \frac{1}{10} \text{ (approximately).}$$

2. Amount of liquid drawn up by average twig:

Experiment performed with water. Twig measuring 5.7 inches and having a wet weight 2.626 gm. took up 1.5 cc. of water.

3. Assume: (a) In all twigs there is a similar proportion of dry weight of bast to wet weight of twig ($\frac{1}{10}$); (b) all twigs will draw up a proportionally similar amount of liquid; (c) all tissues of the stem will receive an equal amount of solution.

4. Carbohydrates used:

Glucose..... $C_6H_{12}O_6$ ..... Unit molecular weight 180
Levulose..... $C_6H_{12}O_6$ ..... Unit molecular weight 180
Saccharose.... $C_{12}H_{22}O_{11}$ ..... Unit molecular weight 342
Lactose..... $C_{12}H_{22}O_{11}$ ..... Unit molecular weight 342
Salicin..... $C_{13}H_{18}O_7$ ..... Unit molecular weight 286

Let 100 gm. of bast tissue be increased by 50 gm. of carbon: then 0.2626 gm. ($\frac{1}{10}$ wet weight twig) will be increased by 0.1313 gm. carbon; twig takes up 1.5 cc. of solution; therefore, 1.5 cc. of solution must contain 0.13 gm. carbon.

72 gm. carbon are contained in 180 gm. glucose and levulose.
72 gm. carbon are contained in 171 gm. lactose and saccharose.
72 gm. carbon are contained in 132 gm. salicin.

0.13 gm. carbon are contained in $\frac{0.13}{4}$ gm. glucose and levulose

0.13 gm. carbon are contained in $\frac{0.541}{24}$ gm. lactose and saccharose

0.13 gm. carbon are contained in $\frac{0.143}{6}$ gm. salicin; therefore 100 cc. of solutions must contain 21.6 gm. glucose and levulose, 20.6 gm. saccharose and lactose, and 15.9 gm. salicin.

In the case of saccharose and glucose some twigs were also suspended so that they dipped into a solution of the preceding strength, in lieu of having it injected. The provision of nitrogen for decreasing the ratio was similarly calculated, a 0.2 molecular solution of potassium nitrate increasing the nitrogen content by 1.6 per cent. In the case of this series, also, a set of uninjected cultures dipping into the same solution was arranged. Cuttings with decreased and increased ratio were set up in Kilner jars prepared as for normal cuttings, and all placed under similar conditions of illumination and temperature in the greenhouse.

Morphological results

Up to the tenth day, when the first samples of bast tissues from the base of the stem were taken, no morphological change was observed, and it was not until after fourteen days that root initials were formed on one of the cuttings dipping into water, and the shoots of the carbon-infused cultures began to open. From that day bud development was rapid in the sugar cultures. On the fifteenth day the order of development was as follows:

Lactose and glucose (almost alike)
Salicin
Saccharose and levulose (no growth).

The next day the buds of the saccharose-infused cuttings showed a slight tendency to bursting, and on the seventeenth day the order was:

Glucose, well developed shoots with root initials established
Lactose, also with root initials
Salicin, no roots
Saccharose, slight growth, no roots
Levulose, no development.

By this time the buds were beginning to unfold in the normal cultures, and roots were also forming. The nitrate cultures showed no sign of development, but exhibited a marked tendency to develop molds. By the twenty-eighth day the difference between the three series of cultures was very striking. The nitrate cuttings were still undeveloped, the normal cultures were well developed, the cuttings suspended in water being further advanced in shoot and less advanced in root development than those suspended in moist air. In the former all roots developed above the water line.

The sugar series was about twice as advanced as the normal cultures, both in root and shoot development. Long shoots with well expanded leaves were shown by the glucose, saccharose, and levulose cultures, which latter, although last in bursting, grew very quickly and surpassed the lactose and salicin cultures. The cuttings hanging into glucose and saccharose showed also well developed shoots but as yet no roots. Six days later roots developed, but at the top of the cutting and above the shoots. This reversed polarity was shown by the cuttings suspended into both glucose and saccharose. Whether this is an accidental peculiarity of these individual cuttings, or whether the difference may be constant, is at present under investigation.

Many of the sugar-infused cultures showed roots all over the stem, that is, poorly marked polarity. The nitrate-infused cultures were badly molded, but a few buds were beginning to develop, and in one case a root had emerged (as in the sugar cultures) at the top instead of the base. This root, the only one developed by the nitrate cultures, grew to about 0.25 inch and then died off. The shoots were extremely backward, and after bursting the bud developed no further (figs. 1, 2).

By the end of the next fortnight, etiolation was strongly marked in all the sugar cultures, which had reached their maximum develop-

ment; less so in the normal cultures; while the normal cuttings dipping into water were fresh and green. This fact is probably related to the greater water supply and the consequent easier translocation of the products of metabolism. Even at this date the nitrate cul-

FIGS. 1–3.—Winter shoots of *Salix viminalis:* fig. 1, injected with KNO_3 (suspended in moist air); fig. 2, normal (suspended in moist air); fig. 3, injected with glucose (suspended in moist air).

tures were still undeveloped, showing very few root initials and hardly any bud growth.

These results are apparently in line with those of Miss REID (22). She grew carbon-high and nitrogen-high cuttings of tomato in light and darkness, and in solutions with and without nitrates, and in every case the carbon-high plants grew rapidly, while the nitrogen-high cuttings showed very poor root and shoot development or none at all.

These morphological observations seem unusual in view of the

facts that nitrogenous manures applied to plants stimulate rapid growth; and that, judging from the results of the experiments on wheat, embryonic and young growing parts are rich in nitrogen; or the explanation of MURNEEK (19) of the inhibition of vegetative growth by fruit setting in the tomato as due to the monopoly of the soluble nitrogen by the fruit and the consequent accumulation of carbohydrate in the vegetative parts. It stresses the fact, however, that it is the ratio of carbon to nitrogen which is of prime importance, and not the amount of either element present.

The explanation of some of these results will be considered later.

Statistical results

Tables I–III show the main trend of the C/N relation during regeneration, and the contrast between the three sets of cultures. Apparently the first stimulus to regenerative germination, combined with the external conditions of moisture and warmth, is the presence of free glucose or lactose, both of which are very easily respirable sugars. Salicin itself is not directly respired, but is turned into glucose before utilization by the willow. Thus cuttings infused with glucose and lactose respired rapidly. Respiration provides the necessary energy for the withdrawing of reserve nitrogenous material from the wood and pith, and their upward translocation. Thus the beginning of growth is marked by an increase in the nitrogen percentage of the phloem tissues. This is most marked under the buds. The C/N ratio falls considerably, due to decreased carbon and increased nitrogen.

The nitrogen drawn into the bast tissues is then translocated upward, so that the tip becomes richer in this element than the base of the shoot. On the other hand, the carbon is withdrawn, more particularly from the upper region, by the increased respiration of the unfolding buds.

Whether a part of the large amount of sugar drawn into the cutting passed into the tissues of phloem, cambium, and cortex is not seen from these analyses, begun ten days after setting up. However, judging from comparisons with the nitrate cultures, and from the results of the more slowly developing saccharose culture, there is a probability that this is the case. In the case of saccharose, after

TABLE I

NORMAL CUTTINGS

	Moist air			Water		
	Carbon %	Nitrogen %	C/N	Carbon %	Nitrogen %	C/N
Undeveloped twig						
Bast tissues	34.479	1.623	21.2	34.479	1.623	21.2
Wood	40.49	1.963	21.7	.....	.....	.....
Bud	31.778	5.735	5.5	31.778	5.735	5.5
10 Days						
Bast tissues	34.18	1.819	18.9	34.23	3.621	9.5
Root initial area, bast	34.55	2.089	17.2	.....	.....	.....
19 Days						
Root area, bast tissues	34.00	1.801	18.8	.....	.....	.....
Young root	28.701	5.941	4.8	.....	.....	.....
Unfolding shoot	30.714	6.391	4.8	.....	.....	.....
Bast tissues around shoot	37.63	3.002	12.5	.....	.....	.....
28 Days						
Base of shoot, bast tissues	33.756	2.917	11.6	.....	.....	.....
Older root	29.106	5.944	4.9	.....	.....	.....
Older shoot	34.624	5.310	6.6	.....	.....	.....
Bast tissues around shoot	34.00	2.914	11.7	.....	.....	.....
37 Days						
Base of shoot, bast tissues	30.221	3.504	8.1	.....	.....	.....
Tip of shoot, bast tissues	27.841	3.793	7.3	.....	.....	.....
Under bud, bast tissues	33.615	2.614	12.9	.....	.....	.....
Wood	36.138	1.514	23.9	.....	.....	.....

TABLE II

NITRATE CULTURES

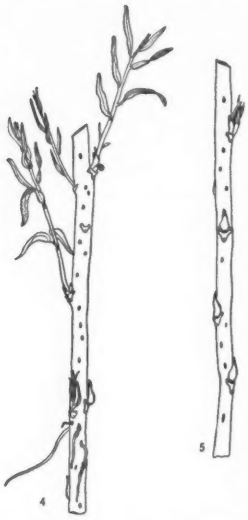
	One-fifth molecular potassium nitrate injected cultures			Hanging into one-fifth molecular potassium nitrate		
	Carbon %	Nitrogen %	C/N	Carbon %	Nitrogen %	C/N
Undeveloped						
Bast tissues	34.479	1.623+ possible 1.6	?	34.479	1.623	21.2
10 Days						
Bast tissues	31.3	2.651	11.8	34.045	(?) 3.794	(?)7.9
19 Days						
Bast tissues	30.764	2.372	12.9	.....	.....	.....
28 Days						
Bast tissues	34.215	3.998	8.5	26.136	1.952	13.5
37 Days						
Bast tissues, base of stem	31.256	2.523	12.4	.....	.....	.....
Bast tissues, tip of stem	30.744	3.699	8.3	.....	.....	.....
Bast tissues, under bud	32.045	3.023	10.6	.....	.....	.....

TABLE III

SUGAR CULTURES

	SACCHAROSE			LACTOSE			GLUCOSE			LEVULOSE			SALICIN		
	Carbon %	Nitrogen %	C/N	Carbon %	Nitrogen %	C/N	Carbon %	Nitrogen %	C/N	Carbon %	Nitrogen %	C/N	Carbon %	Nitrogen %	C/N
Undeveloped Bast tissues	34.479 + (possible 50)	1.623	?	34.479 + (possible 50)	1.623	?	34.479 + (possible 50)	1.623	?	34.479 + (possible 50)	1.623	?	34.479 + (possible 50)	1.623	?
10 Days Bast tissues	37.38	2.409	15.5	31.531	2.105	15.0	33.545	1.881	18.6	32.925	1.929	17.3	33.579	2.102	15.9
19 Days Bast tissues															
base of stem	32.082	2.155	14.9	31.534	2.130	15.0	31.595	1.99	15.8	32.89	2.339	14.0	34.512	2.741	12.6
top of stem	28.078	2.724	10.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
under bud	34.228	2.548	13.6	30.058	3.247	9.4	31.750	2.132	15.0	28.161	3.292	8.6	.....	.....	.....
28 Days Bast tissues	28.9	2.974	9.6	31.945	1.336?	2.4?	36.759?	2.132	18.2?	22.944	1.89	12.1	33.33	3.142	10.6
37 Days Bast tissues															
base of stem	30.7	3.088	10.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
top of stem	28.00	3.288	9.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
under bud	29.409	3.392	9.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

ten days the carbon percentage of the bast tissues had risen from 34 to 37 per cent. This is interesting since in fruit trees (for example, apple) saccharose is looked upon as a special form of storage, with special functions at bud-break. The saccharose and levulose cultures at first were slower in developing, presumably due to greater difficulty in the respiration of these sugars. However, once the C/N ratio had been lowered to within the growing limits (ratio probably below 15), growth of these two cultures was vigorous and rapid.



FIGS. 4, 5.—Winter shoots of *Salix viminalis:* fig. 4, suspended in water; fig. 5, suspended in one-fifth molecular KNO_3.

In the normal cultures respiration was much slower, and in ten days only 1 per cent of the carbon had been respired. This meant that the nitrogen translocation was also slower, and it thus took longer for the "growth ratio" to be established. The cuttings hanging in water contained after ten days much more nitrogen than the other normal cuttings, an abundant supply of water evidently being an important factor in the translocation of this element into the phloem. The low C/N ratio so produced favors shoot formation rather than root development.

In the case of the nitrate cultures the injected nitrate passed into the bast tissues, but the carbon content of the bast tissues was not lowered by respiration. The cutting had evidently been thrown into Class I of KRAUS and KRAYBILL (10), the nitrates being excessive; and, in contrast with the case of respirable carbon compounds, the plant has no means of removing them, so growth was checked.

As in the case of flowering, so in the regenerative germination it is the ratio of the C/N which is all important, and not the relative amount of either element.

One question which cannot be avoided is where the nitrogen in the new growths comes from. Chemical analysis shows that in part

it is withdrawn from the wood and pith, but since the percentage is almost doubled, even after rapid growth has taken place, it is doubtful if the amount in the wood and pith is sufficient to cause such an increase. Can the nitrogen of the atmosphere play some part in green plant metabolism after all?

The young roots are rich in nitrogen and somewhat low in carbon, and as they develop, the normal increase in the C/N ratio is followed. The undeveloped buds contain a very high percentage of nitrogen, and the lowest C/N ratio of the whole cutting. As they develop, the nitrogenous material is piled up in the tissues around the base of the growing shoot, so that a low C/N ratio is maintained by the shoot for a considerable time.

FIGS. 6, 7.—Winter shoots of *Salix viminalis:* fig. 6, suspended in saccharose; fig. 7, suspended in glucose.

New polarity hypothesis

In the mature long stem, while attached to the branch of the tree, there is relatively little gradation in either carbon or nitrogen, although the percentage of the latter element is slightly higher in the apical portion. On severing the stem into portions and hanging each portion in moist air in a warm room, activated respiration seems to supply energy to expedite the translocation of nitrogenous compounds. The nitrogen is translocated upward, the carbon flow being downward. Thus in any cutting prior to growth a lower C/N ratio is established at the apex, with a higher ratio at the base and a gradation in between. Shoots

are formed in the region of the lowest C/N ratio; roots can and do form where the higher ratio occurs.

This gradation in C/N ratio can be translated into terms of LOEB'S "root forming and shoot inhibiting" hormone (a high C/N ratio), and "shoot forming and root inhibiting" hormone (a low C/N ratio). The absolute values of the elements are of little importance compared with the ratio, so that severing a stem leaves the gradient of the ratio in each piece unaffected.

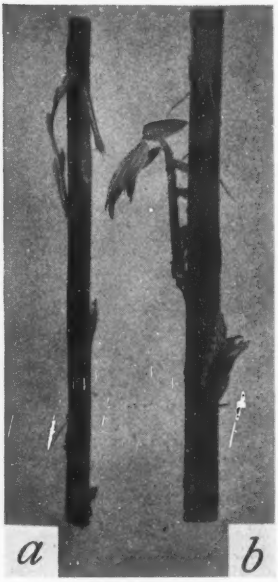


FIG. 8.—Shoots: *a*, normal (suspended in water); *b*, sugar (suspended in glucose).

When a cutting is inverted, a lag is necessitated, possibly owing to the reversed position of the vessels of the wood and bast, but the vessels maintain their normal action, the nitrogen flow now being downward, the carbon upward. Thus the morphological base has the highest C/N ratio and bears roots; the true apex with a lower C/N ratio produces shoots.

If the shoot is maintained in the horizontal position normal polarity is maintained, since translocation of nitrogen is not interfered with. The influence of the leaf upon root formation as reported by LOEB (13) can also be explained on this basis. LOEB states that if on a horizontally suspended stem of *Bryophyllum* (in which the growing point is cut off) one leaf is preserved at the apex, strongly marked geotropic curvature occurs with extensive root formation at every node of the stem.

If the single leaf is basal the curvature is considerably less, however, and the little root formation which occurs is confined to the nodes of the basal leaf and the internode below. These effects can be altered by incisions, or by removing the cortex on one side of the stem. This is explained in the following way.

The respiratory activity of the leaf appears to create a suction

upon all the tissues below it. Thus the ratio of carbon to nitrogen in these tissues is reduced by carbon loss. The pull of transpiration, too, causes the nitrogen to rise in the stem. Thus the C/N ratio gradient is maintained, while the ratio itself below the leaf is lowered, so that growth of roots is established. Again, since a lowering of the C/N ratio means changing the plant into a chemically younger state; geotropic curvature is thus increased, younger tissues being more responsive than mature ones.

If the leaf is at the base, however, only the portion below it will experience the suction, due to respiration and transpiration, and only this part will be turned into a regenerating region. The tissues of the stem above the leaf are thus chemically old, and hence less responsive to geotropism. Incisions naturally affect the response since they restrict the upward flow of nitrogen.

This hypothesis can also be used to explain the production of roots at the apex of the cuttings suspended in sugar. In this case the shoots developed first, and transpiring actively drew up the sugar solution which necessarily accumulated at the top of the cutting. Thus the C/N ratio became highest at the top, and roots consequently developed in that area. This assumption, unlike the primary hypothesis, is not supported by chemical statistics, but is at present under investigation.

These results are not in any way complete: more abundant data are required at more frequent time intervals, particularly at the beginning of the experiment, but they are submitted as a preliminary outline of the relation of the C/N ratio to regeneration.

Summary and conclusions

1. Experiments on the regeneration of cuttings of *Salix viminalis* have been carried out with normal cuttings, and with cuttings injected with potassium nitrate and various sugars.

2. The initiation of growth is due to stimulated respiration, giving energy for the withdrawal of nitrogen into the bast, and its consequent upward translocation, particularly to the buds. The injected sugars hasten this considerably, especially easily respirable

sugars such as glucose and lactose. Potassium nitrate in excess prevents the commencement of growth.

3. LOEB's hormone hypothesis is explained as a gradation of the C/N ratio throughout the cutting. Shoots grow at the area of the lowest C/N ratio, roots at the higher. The effect of a leaf at the apex or base of the shoot is also interpreted on this basis.

This work was carried out in the botanical department of the University College of South Wales, Cardiff, under the direction of Professor MCLEAN, to whom I wish to express thanks for his interest and suggestions during its procedure.

UNIVERSITY COLLEGE OF SOUTH WALES
CARDIFF, S. WALES

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EFFECTS OF ULTRA-VIOLET RADIATION ON VARIOUS FUNGI

F. L. STEVENS

(WITH TWELVE FIGURES)

Introduction and general methods

As announced in a preliminary note,[1] ultra-violet radiation has a striking and profound effect upon the reproductive processes in various fungi, calling forth immediate and profuse sexual or conidial development in fungi that normally exhibit these stages only very rarely. The present article gives in some detail the studies upon which the preliminary report was based, as well as those of subsequent experiments.

The method of procedure unless otherwise stated was as follows: Full radiation from a Cooper-Hewitt quartz mercury arc operated at 4.5 amp. and 66 volts was employed. The agar plate cultures with the Petri dish covers removed were placed directly in front of the source of light, at a distance of 21 cm. A shield of hard rubber from a photographic plate holder was placed in front of the plate, in order to shade certain portions of the colony, usually one-half, from the light.

Difco corn meal agar was employed, 10–12 cc. per Petri dish. Inoculations in early experiments were made by transferring a bit of mycelium-bearing agar to the center of a solidified plate. Later it was found to be more expeditious and satisfactory to make a suspension of conidia in a drop of sterile water and transfer an oese of this suspension to the agar plate. Cultures used in the experiments were 4–7 days old.

I wish to acknowledge my great indebtedness to the Department of Physics of the University of Illinois for the use of apparatus, and to Professor JACOB KUNZ and F. W. COOKE for suggestions and helpful assistance.

[1] The sexual stage of fungi induced by ultra-violet rays. Science N.S. 1923.

Glomerella cingulata, strain G 10

This strain, *Glomerella cingulata* (Stoneman) Spaulding & von Schrenk, strain G 10, originally isolated from apples affected with bitter rot, was most extensively used in these studies, and the following account is based upon experiments with it. A single conidium was isolated in October, therefore G 10 as treated in these accounts is of monosporous origin. Normally as cultured on agar it develops luxuriantly at the rate of about 4 mm. per day radially, thus completely occupying a Petri dish in about 13 days. G 10 in one large series of cultures kept under observation until 30 days old was not seen to produce perithecia in the monosporous strain on agar or apples. In very old cultures the original strain did occasionally bear a few sclerotioid masses 2–3 mm. in diameter, which produced numerous perithecia. Such cultures closely resembled those figured by SPAULDING and VON SCHRENK, except that the perithecial sclerotia were much less numerous. The cultures also differed from their descriptions in that the perithecia were not imbedded in a stroma, but rather consisted of numerous, nearly globose perithecia of neat contour, closely grouped, but which were readily separated by crushing the mass.

Conidia, when placed upon agar, showed but the slightest indication of germination in 6 hours. In 22 hours, however, they had developed filaments 0.7 mm. long along the surface of the agar, and 0.8 mm. long penetrating into the agar. Thus in agar 1 mm. deep 24 hours suffices for the mycelium to penetrate to the glass below.

EFFECTS OF ULTRA-VIOLET RADIATION

When colonies 2–7 or 8 days old are radiated with the appropriate dosage, death and collapse of the aerial mycelium is immediate, growth of the colony is temporarily stunted, and perithecial formation is induced both by the direct and by diffused rays.

LETHAL EFFECTS.—To determine the lethal dosage upon spores, two series of 14 plates each were arranged: one with the spores placed upon the top of the agar, the other with the spores covered by the agar, the agar layer being approximately 1.5 mm. thick. Each series was radiated with exposures varying from 5 to 90 seconds. With a treatment of 5 seconds only a few of the unprotected

spores germinated and these but poorly. With an exposure of 10 seconds still less germination was noted, although in both instances vigorous colonies resulted. With an exposure of 15 seconds or more no germination resulted. The spores protected by the agar layer, subjected to an exposure of 5, 10, and 15 seconds, gave good germination; 20, 25, and 30 seconds gave fair germination; 35, 40, 45, and 50 seconds gave poor germination; 60, 70, and 80 seconds gave very poor germination; 90 seconds gave still fewer germinating spores, but still sufficient to result in a vigorous colony.

The minimum lethal dosage for uncovered spores, therefore, lies somewhat over 10 seconds but less than 15 seconds. The lethal dosage under 1.5 mm. of agar lies at slightly more than 90 seconds.

The lethal effect on the aerial mycelium is at once evident, especially in regions bearing considerable floccose mycelium, in that all such mycelium immediately falls flat upon the agar, thus giving the radiated half of the colony a strikingly different appearance from that of the non-radiated portion.

COLONY STUNTING.—Colony stunting is most clearly evident about 20 hours after radiation, the colony then appearing as though no growth had occurred subsequent to exposure on the radiated half, while it had proceeded normally on the non-radiated half, thus making the colony radius on this half 3–4 mm. longer than on the other half. Shortly growth is resumed by the deeper, less injured mycelium, and in a few days macroscopic evidence of the stunting is lost.

PERITHECIAL FORMATION.—Direct radiation, that is, that on the uncovered half of the colony, resulted in very numerous perithecia either superficial or buried, depending upon the dosage (figs. 1, 2).

Diffuse radiation, that is, the rays deflected by some irregularity of surface so that they reached under the protective shade employed, also gave abundant perithecia, either superficial or deep. Since even with very long exposures to direct radiation certain regions received very weak doses by diffusion, perithecia were produced by diffusion in exposures much too strong to give them by direct radiation.

FORMATION OF ACERVULI.—Acervuli were distinctly more abundant in the non-radiated half of a plate following radiation of one minute through vitaglass; also short dosages of 0.25–0.75 of a second

of full radiation increased conidial production on the non-radiated portions of this colony.

Dosage

With exposures of 0.5–1 second no colony stunting was apparent; 2, 3, and 4 seconds gave slight stunting. All exposures from 5 seconds to 15 or more minutes produced distinct colony stunting.

The aerial mycelium was killed by 15 seconds. With exposures of 1–4 minutes very abnormal branching was induced, this effect increasing with the dosage; with 1.5 and 2 minutes' radiation the

Figs. 1, 2.—Fig. 1, *G. cingulata* 8 days old radiated one-half second; numerous perithecia by direct radiation, none by diffuse radiation; fig. 2, *G. cingulata*, radiation 5 seconds; many buried perithecia by direct rays, few by diffuse rays.

stunted regions were perceptibly darkened. With exposures of 4–15 minutes the lethal effect was much greater, and numerous sclerotioid masses developed; and new centers of growth, evidently originating from deep cells that escaped the deadly rays, resulted in fan-shaped areas.

All plates exposed 1–15 minutes showed in the shielded half of the colony, close to the line of shielding, numerous perithecia. When the exposure was short (1–4 minutes) these perithecia were found very close to the shadow line, 1 mm.; given an exposure of 5–15 minutes, they were further from this line (2–3 mm.). A typical distribution is shown in fig. 2. Many of these perithecia are superficial, but some are deep in the agar (1 mm.). They vary greatly in size,

both large and small being found irregularly distributed both laterally and vertically throughout the agar.

Such perithecia are the result of rays reflected from irregularities here and there on the surface of the medium or other surroundings, and the irregularity in their size and location is due to variation in intensity of those irregularly reflected rays. The absence of perithecia close to the shadow line in heavy dosage is presumably due to a lethal intensity in this region. An occasional isolated perithecial region in the shadow, in the case of long exposure, is explained as caused by rays from some special irregularity in the reflecting surface.

A series of 4-day-old colonies was radiated with the following dosages: 1, 2, 5, 15, 30 seconds, 1, 2 minutes. The one second radiation as well as all the others induced numerous surface perithecia by diffusion. Direct radiation of 5 seconds or longer gave buried perithecia, although smaller dosage did not. The long exposures of 1 and 2 minutes gave fewer buried perithecia than did shorter exposures. Repetition with dosages of 0.5, 1, 2, 3, and 4 seconds resulted in many surface perithecia by direct radiation, mainly on the inner zone of the colony. One, 1.5, and 2 minutes gave many surface perithecia by diffusion; 1.5 and 2 minutes gave few and irregularly located deep perithecia by direct radiation.

A series of 4-day-old colonies was exposed at 230 cm. from the source of radiation for 10, 20, 30, 40, 50 seconds, 1, 1.5, 2, 5, and 10 minutes. This distance gives approximately the same energy exposure that one at 21 cm. would give in one one-hundred-twentieth of the time. Ten and 20 seconds gave negative results; 30 seconds gave surface perithecia in the oldest colony zone; 40 and 50 seconds gave the same result but intensified; 1, 1.5, 2, 5 and 10 minutes (=5 seconds at the usual distance) gave both superficial and deep perithecia.

Slight colony stunting was evident at one minute and longer at 230 cm. (=0.5 second at the usual distance), distinct stunting at 10 minutes (=5 seconds at the usual distance). Various exposures in diffused light, as from the wall, from a metal sheet, etc., gave only negative results.

The conclusions as to dosage and perithecial formation may be

summarized as follows: Direct very weak dosage gave superficial perithecia; stronger dosage gave perithecia on the surface and deeper; still stronger dosage gave only imbedded perithecia; still stronger dosage gave no perithecia. When obvious colony stunting occurs the dosage is too great for the development of surface perithecia.

Relations of agar depth

Agar plates were so tilted before solidification that the agar was shallow at one side of the plate and deep at the opposite side. Four-day-old colonies on these plates were radiated for one minute. Diffuse radiation induced superficial perithecia as usual on both the deep and the shallow agar. Many buried perithecia developed in the deep agar but none in the shallow, evidently owing to overdosage.

In a series of plates of deep agar (3 mm.), with radiation varying from 0.5 second to 5 minutes, the one-half-second direct dosage gave only surface perithecia, none by diffusion; 0.5 minute gave surface perithecia by diffusion; under direct radiation all were buried from 1 to 2 mm. deep in the agar; with 1, 2, 3, and 5 minute exposures they were buried still deeper (2–3 mm.), that is, in the lowest layer of the agar; the 3 and 5 minute dosages, however, gave but few perithecia.

Age susceptibility to perithecial production

Colonies more than 4 days old when radiated developed perithecia in all of the exposed area, even from the youngest mycelial threads, but perithecia were often more abundant in mycelium only one day old than in older mycelium. Sometimes the one-day-old zone of the colony appeared as a dark band, due to the profusion of perithecia; in other cases the whole colony was equally perithecial. Colonies as young as 2 days old produced perithecia, but were not so satisfactory for study as were older colonies. Colonies 12 days old, on radiation, gave only a few perithecia in the 11- to 12-day-old regions, most occurring in the 5- to 9-day-old regions.

Effect of quality of medium

Plain agar, that is, merely agar and water of the usual percentages without addition of nutrients, gave scant growth, and on radiation with various dosages gave no perithecia, probably due to lack

of sufficient vigor of growth to support perithecial formation. With the addition of corn meal agar to less than 50 per cent strength, poor growth and no perithecia resulted upon radiation. On corn meal agar of 50–70 per cent nutrient strength abundant perithecia developed on radiation.

Possible chemical changes as causal

To attempt to determine whether or not the radiation caused some chemical change in the medium favorable to perithecial production, two trials were made as follows: (1) A plate of sterile corn meal agar was radiated 3 minutes, then inoculated with G 10. (2) A plate of sterile corn meal agar was radiated 3 minutes, and a piece of this agar about 4×4 cm. lifted out and laid upon a 4-day-old colony covering about half of it. Growth in both cases was normal, with no perithecial development, indicating the absence under these conditions of any chemical capable of inducing perithecial formation.[2]

Heredity

That the sexogenetic effect is local is shown by the fact that the new mycelial growth is normal and non-sexual, although it arises directly from a mycelial region that is most strongly perithecial; that is, the perithecia are produced only on the mycelium actually stimulated, not on the progeny of these cells. Also, in numerous instances, transfers to new plates were made from regions of heavy induced perithecial formation. All such cultures behaved as did ordinary non-radiated cultures.

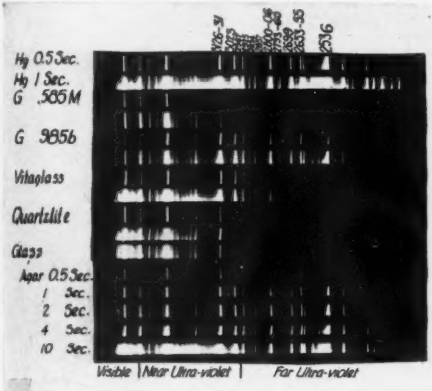
Two series of 8-day-old cultures were radiated for 5, 30, and 60 seconds, and were (1) inoculated from an induced perithecial region; (2) inoculated from normal non-perithecial mycelium. No difference in perithecial development was apparent between these two series, indicating that the induced ability to produce perithecia was not passed on to the progeny under the conditions of the experiment. It is to be noted, however, that in such tests as those of hereditary effects, it is possible that negative results were due to the fact that the mycelial cells that were tested, although radiated, had not responded to the stimulation. The cells actually known to have

[2] See also Bovie, Bot. Gaz. 61:1. 1916; and Coblentz and Fulton *l.c.*, p. 663.

responded are those that give rise to perithecia, and a test of the perithecium-producing capacities of the ascospores will therefore be of especial interest.

Efficient wave lengths

To determine the actual wave lengths responsible for perithecial production, screens were used as follows:



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4

Figs. 3, 4.—Fig. 3, spectrogram showing various absorptions; fig. 4, spectrogram printed in perithecia; region represented is from 302 to approximately 180 mμ.

(1) A blue-purple glass 7 mm. thick, known as G. 985 *b* and also as Corex, gives the absorption spectrum shown in fig. 3; that is, it lets all wave lengths pass down to approximately 246 mμ.

(2) Vitaglass 2 mm. thick excludes more of the shorter waves, fig. 3.

(3) Window glass 2.3 mm. thick allows no waves shorter than the bands 312–3 mμ to pass.

(4) Quartzlite differs from window glass only in that it permits the passage of the next band, 302 mμ.

The effect of the corn meal agar 1 mm. thick is also shown in

fig. 3. It is to be noted that with short exposures, 0.5–5 seconds, there is considerable absorption of the shorter waves, but 10–30 seconds suffices to give a very effective exposure even through this medium.

The sexogenetic effect and colony stunting were evident in all trials where Corex or vitaglass was used, but never appeared when window glass was used.

A comparative series of experiments with radiation through vitaglass and Corex for 30 seconds on colonies ranging from 2 to 8 days old showed perithecia throughout the exposed area. No essential difference between the two modes of radiation appeared, except that there was a much larger perithecial area by diffusion from Corex than from the vitaglass, due to the irregular surface of the Corex screen.

A second series with the same screens, with dosages of 10, 20, 30 seconds, 1, and 1.5 minutes showed no difference in sexogenetic effect; many buried perithecia were produced in all exposed regions of the colony. The two groups of rays do, however, produce slight differences in the contour and color of the mycelium, and consequently in the general appearance of the colony.

The conclusive evidence as to wave lengths effective in stimulating perithecial formation was given by the Hilger Quartz Spectrograph E2. To use this instrument two glass strips, 15×2.5 cm., were placed on the bottom of a 16 cm. Petri dish 1 cm. apart, and the whole sterilized by hot air; then sufficient agar was poured in the dish to cover the glass strips to a depth of 1 mm. When solidified a row of spores was placed on the agar midway between the glass strips. This resulted, in 3 or 4 days, in a growth of susceptible mycelial threads over the entire surface of the two glass strips. These glass strips were then cut out and attached to a glass plate and placed in the plate holder. Thus, when placed in the spectrograph, the fungus growth rested exactly in the position occupied by the photographic film in ordinary exposure. The slit was adjusted at 0.75 mm. width and 1 cm. long. This gives images of the spectrum lines 1 cm. long and 1–2 mm. wide.

The first trial with an exposure of 2 hours was too long. It resulted in collapse of the aerial mycelium and in non-perithecial regions coincident with all of the more energetic bands throughout the

whole ultra-violet region down to 180 mμ, beyond which the spectrograph did not reach. By diffusion, the whole of the space between all of these bands was occupied by buried and surface perithecia.

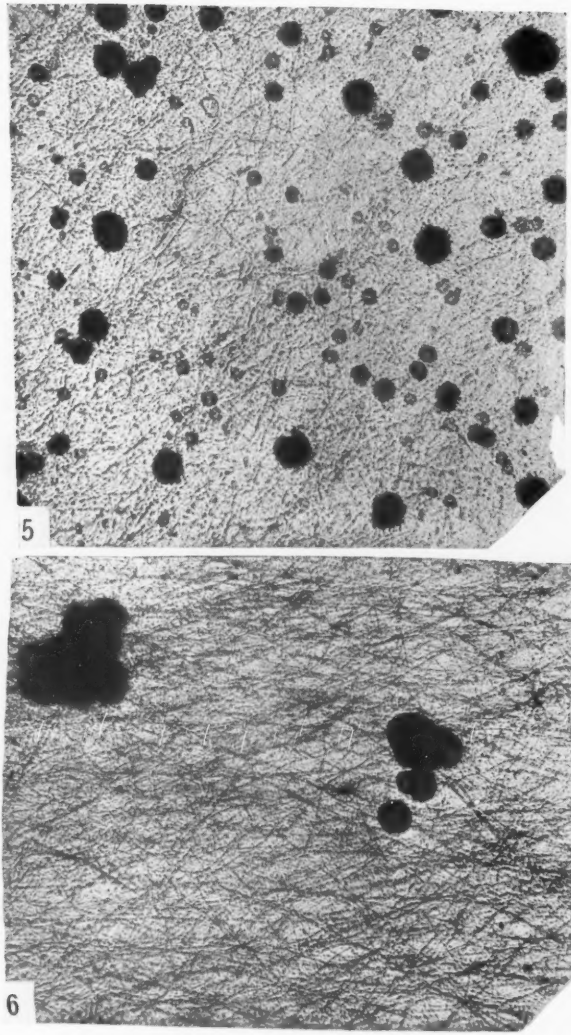
Repetition with exposures of 3, 15, 30, and 60 minutes all resulted in the death of the aerial mycelium, more pronounced with the increase of exposure, but in all cases in the development of buried perithecia throughout the whole range of the ultra-violet spectrum. Such a spectrogram printed in perithecia is shown in fig. 4. Similar trials with various separate regions of the spectrum were made. Thus the band 254 mμ, used alone, gave abundant perithecia with the 3- and 15-minute exposure, but none with 1-minute exposure. The four bands 3021, 3023, 3026, and 3027 Ångstrom gave similar results. In another exposure of 15 minutes the whole range of the spectrum from 254 mμ to 180 mμ was used, resulting in perithecia throughout the range. From all the evidence, it appears that the effective rays lie throughout the region of wave lengths shorter than 313 mμ.

Other strains of G. cingulata

Of the several other strains of *G. cingulata* experimented with, nos. 2, 7, and 28 gave essentially the same results as did G 10. No. 9 behaved essentially like G 10, with the striking exception that the perithecia were produced in clumps, not scattered (figs. 5, 6, 7). No. G 9 on agar plates without radiation sometimes produced a few comparatively large (2–3 mm.) sclerotial masses on which quite numerous perithecia were borne. Nos. 8, 16, 17, and 30 produced no perithecia, but did produce acervuli on the lower, not on the upper surface of the agar, and not in the non-radiated regions (fig. 8). On no. 19 sclerotial formation was inhibited and conidial formation stimulated (fig. 9). No. 20 produced perithecia, but only in the oldest zone. Strains 4 and 19 gave only negative results as regards perithecia at 1, 15, and 60 seconds, but did produce acervuli on the mycelial tips radiated.

Summary regarding Glomerella cingulata under full direct radiation at 21 cm.

1. Some spores are prevented from germination by exposure for 5 seconds, and all are killed or prevented from germination by 15



FIGS. 5, 6.—Fig. 5, G 10 showing scattered perithecia; fig. 6, G 9 showing perithecia in groups, not scattered.

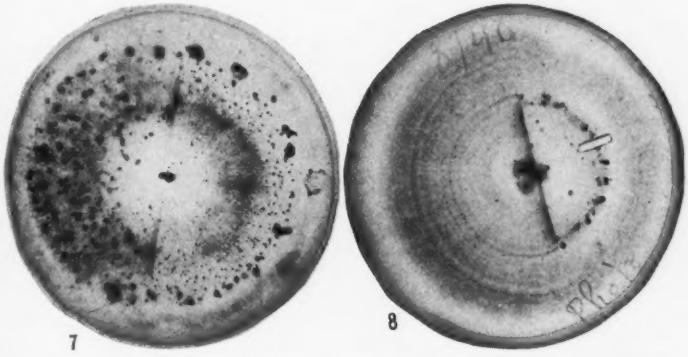
seconds' exposure; more than 90 seconds are required to kill all spores when covered by 1.5 mm. of corn meal agar.

2. The aerial mycelium is killed by 15 seconds' exposure.

3. Distinct colony stunting is produced by 5 seconds' exposure, and slight stunting by 2–4 seconds.

4. Diffuse radiation produces effects like those of much weaker direct radiation.

5. Direct radiation in mild dosage (0.25–4 seconds) resulted in surface perithecia; in larger dosage (5 seconds to 2 minutes) in buried perithecia.



FIGS. 7, 8.—Fig. 7, G 9, radiation 10 seconds; fig. 8, G 8, radiation 40 seconds; no perithecia but numerous acervuli in radiated portion.

6. With sufficient depth of agar much greater dosage may be used.

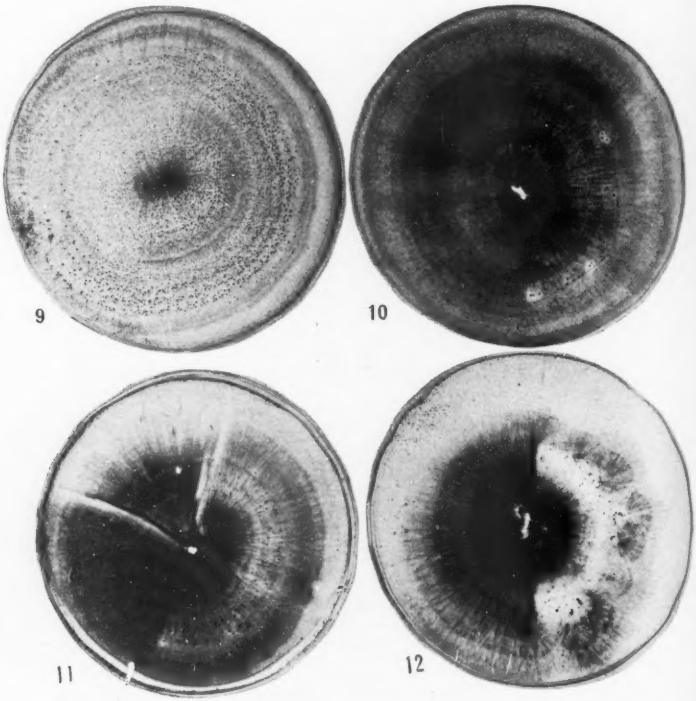
7. The longer the exposure the deeper in the agar lie the perithecia.

8. The rudiments of perithecia may be recognized in 15 hours, probably much earlier. The perithecia bear mature asci and ascospores in 4 days.

9. The youngest mycelium is most easily killed, and is most responsive in perithecial production; the older mycelium is less sensitive in both regards.

10. A medium poor in nutrients does not render a colony capable of producing perithecia on radiation.

11. The production of acervuli in the non-radiated parts of the colony is largely increased by radiation.



FIGS. 9–12.—Fig. 9, G 19, radiation 15 seconds; conidia produced abundantly at tips of mycelium radiated and sclerotial formation inhibited; fig. 10, *Coniothyrium*, radiation 10 seconds; many pycnidia appear at points occupied by tips of mycelial growth at time of radiation; fig. 11, *Coniothyrium*, radiation 30 seconds; many buried pycnidia in two outermost zones; fig. 12, *Coniothyrium* 8 days old, radiation 3 minutes; many buried pycnidia in oldest zone, much lethal action in youngest zone, with fanlike new growths originating from surviving centers (these centers also pycnidial).

12. There is no evidence that chemicals produced by the radiation of the medium are responsible for the effects noted. The effect is extremely local and apparently is not hereditary.

13. Different races of *G. cingulata* respond differently to radiation.

14. The efficient rays lie throughout the region of the ultra-violet of wave lengths shorter than 313 mμ.

Coniothyrium sp.

An unknown species of *Coniothyrium* which accidentally appeared upon plates in the laboratory was subjected to radiation. This fungus normally never produced pycnidia until the culture was very old, completely filling the Petri dish. It then usually, but not always, produced a circle of pycnidia at the edge of the dish.

Coniothyrium colonies 8 days old were radiated from 1 second to 3 minutes. With 1 second there was very slight, and at 5 seconds distinct, colony stunting but no other perceptible effect. With 10 seconds the extreme tips of the mycelium that was radiated produced numerous pycnidia, some superficial, some buried, so that a nearly perfect arc was formed by these structures (fig. 10). There were also a few scattered pycnidia throughout the zone of mycelium that was 1 to 2 days old when radiated. These were distinctly visible and well formed at 3 days, and must have been recognizable microscopically at 2 days after radiation.

With 30 and 40 seconds the result was similar, except that all pycnidia were buried and that they were more numerous in the 1- and 2-day-old region (fig. 11). With 1- and 2-minute radiation the effect differed in that some pycnidia were formed in the still older regions; with 3 minutes there was much killing of the mycelium in the 1- and 2-day-old zone, and many pycnidia were found in the oldest zone. In the youngest zone a few regions lived through the radiation and became the centers of new growth (fig. 12). The mycelium that remained alive in these centers also developed pycnidia.

From these results it appears that the youngest mycelium is most susceptible to killing and to pycnidial formation; the older less so.

General considerations

In both the various strains of *Glomerella cingulata* and *Coniothyrium*, radiation almost instantly initiates the development of reproductive structures in great numbers where they would not have occurred without radiation, but in no case were structures initiated that were not known to be occasionally produced by these fungi in

the natural course of events. The *Coniothyrium* normally produced but few pycnidia and these after long continued growth. On radiation they were produced at once and in quantity. Certain races of *G. cingulata* that normally produced but few perithecia and these in old colonies, on radiation produced them in great quantities at once. Other races of *G. cingulata* that have not normally been seen to produce perithecia did not yield perithecia on radiation, but even these gave a greatly increased yield of acervuli.

That ultra-violet radiation can induce deep seated changes within the cell, in chromosome number and in polarity is known.[3]

References to stimulation due to ultra-violet radiation below lethal dosage have been made. Thus BROWNING and RUSS[4] reported that bacteria lying between two regions that had been radiated appeared to be stimulated. BOVIE[5] states that if the decomposition of the protein molecule is not carried too far there is cell stimulation.

It appears possible that the effects noted may be due to a stimulus acting either upon the protoplasm or even upon the nuclei; or it may rest either in the production or destruction of some substance within the cell which has direct and specific effect upon cell activity. On the other hand, it is possible that these responses may be considered as in conformity to the laws of KLEBS, that a well nourished organism suddenly inhibited in growth turns to reproduction.[6] However, it is to be noted that while this particular stimulus by definite bands of rays calls forth these responses, no other known agency causing the cessation of growth (and many such have been tested) does call forth these responses. Moreover, the effect is almost if not quite immediate, which makes it difficult to regard the phenomenon as merely a reaction to inhibited growth. Furthermore the action is sharply localized, only the cells impinged upon and not all of these respond. If the cause rested merely upon starvation the results would presumably be more widely distributed among the adjacent mycelial cells.

[3] See JUST as cited by W. C. CURTIS, Science N.S. **67**:141–149. 1928.

[4] Archives Radiology and Electrotherapy **18**:85. 1918.

[5] BOVIE, *l.c.* See also COBLENTZ, W. W., and FULTON, H. R., Sci. Pap. Bur. Standards. no. 495. 1924 (p. 647).

[6] Jahr. Wiss. Bot. **53**:72–73. 1900.

It is of interest to note that there is practically no solar radiation at sea level of less wave length than 305 mμ transmitted through the atmosphere; therefore these fungi in the course of their evolution have not had to contend with or adapt themselves to such influences, although they have had to do so as regards wave lengths longer than 305 mμ.

In addition to any theoretical interests that these phenomena may have, their discovery also serves a practical scientific end, in that by means of ultra-violet radiation we now have a means of inducing largely increased sexual, pycnidial, or other conidial formation; and we can produce either perithecia (*Glomerella*) or pycnidia (*Coniothyrium*) under such conditions that the age of these structures may be definitely known, thus rendering cytological and morphological studies much more definite and trustworthy.

UNIVERSITY OF ILLINOIS
URBANA, ILL.

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SYMBIOSIS IN A DECIDUOUS FOREST

III. MYCORHIZAL RELATIONS

W. B. McDOUGALL AND CHARLOTTE LIEBTAG

The deciduous forest in which these symbiosis studies are being made is known as the University Woods, and is located near Urbana, Illinois. It was described in the first paper of the series.[1] According to the counts there reported, the forest contains 183 species of seed plants. We have examined the roots of 145 of these species, or approximately 80 per cent, to determine the presence or absence of mycorhizal fungi. The method used has been to collect pieces of the smaller roots from two individuals of each species. The roots from each individual were separated into two lots, imbedded, sectioned, and two slides made from each lot. In all cases some sections were cut near the root tip and others in the region of cell maturation. We thus have eight slides from each species, each slide bearing sections from one, two, or three roots, making a total of nearly 1200 slides. The slides were then examined microscopically for mycorhizal fungi. In many cases a binocular microscope with oil immersion lens was necessary to reach a decision, but both writers have examined all slides and agreed in the observations. The results of the observations follow, the nomenclature and arrangement into families being that of GRAY'S *New manual of botany*, 7th ed.

GRAMINEAE:

Elymus striatus, endotrophic mycorhizas.

Hystrix patula, no fungus observed.

Poa sylvestris, no fungus observed.

CYPERACEAE:

Carex grayii, no fungus observed.

ARACEAE:

Arisaema dracontium, endotrophic mycorhizas.

Arisaema triphyllum, no fungus observed.

COMMELINACEAE:

Tradescantia pilosa, endotrophic; fungus not abundant.

[1] BOT. GAZ. 73:200–212. 1922.

LILIACEAE:

Polygonatum commutatum, endotrophic; fungus scarce and often disintegrated.

Smilax herbacea, endotrophic; fungus scarce.

Smilax hispida, no fungus observed.

Smilicina racemosa, endotrophic mycorhizas.

Trillium recurvatum, no fungus observed.

Uvularia perfoliata, no fungus observed.

IRIDACEAE:

Iris hexagona, endotrophic; fungus not abundant.

ORCHIDACEAE:

Aplectrum hyemale, endotrophic; in addition to usual endophyte there is present in some roots a coarse, septate mycelium showing clamp connections; probably a casual parasite.

SALICACEAE:

Populus deltoides, ectotrophic mycorhizas.

JUGLANDACEAE:

Carya cordiformis, some endotrophic and some ectotrophic mycorhizas.

Carya laciniosa, endotrophic; some roots contain a septate mycelium similar to that observed in *Aplectrum hyemale*.

Juglans cinerea, endotrophic; vesicules observed.

Juglans nigra, endotrophic; fungus scarce and much disintegrated.

BETULACEAE:

Carpinus caroliniana, ectotrophic mycorhizas.

FAGACEAE:

Quercus imbricaria, ectotrophic mycorhizas.

Quercus macrocarpa, ectotrophic mycorhizas.

Quercus muhlenbergii, ectotrophic mycorhizas.

Quercus rubra, ectotrophic mycorhizas.

URTICACEAE:

Celtis occidentalis, endotrophic; fungus rather indistinct.

Humulus lupulus, endotrophic; a septate mycelium similar to that observed in *Aplectrum* is present in addition to the usual endophyte.

Laportea canadensis, no fungus observed.

Morus rubra, endotrophic mycorhizas.

Pilea pumila, endotrophic mycorhizas.
Ulmus americana, no fungus observed.
Ulmus fulva, endotrophic mycorhizas.

ARISTOLOCHIACEAE:

Asarum canadense, endotrophic mycorhizas.

POLYGONACEAE:

Polygonum scandens, no fungus observed.
Polygonum virginiana, no fungus observed.
Rumex crispa, no fungus observed.

PHYTOLACCACEAE:

Phytolacca decandra, no fungus observed.

PORTULACACEAE:

Claytonia virginica, no fungus observed.

RANUNCULACEAE:

Actaea alba, no fungus observed.
Anemone canadensis, endotrophic; fungus scarce.
Hepatica triloba, endotrophic mycorhizas.
Isopyrum biternatum, no fungus observed.
Ranunculus septentrionalis, no fungus observed.
Thalictrum dioicum, endotrophic mycorhizas.

ANONACEAE:

Asimina triloba, endotrophic mycorhizas.

MENISPERMACEAE:

Menispermum canadense, no fungus observed.

BERBERIDACEAE:

Caulophyllum thalictroides, no fungus observed.
Podophyllum peltatum, endotrophic; vesicules present.

LAURACEAE:

Benzoin melissaefolium, no fungus observed.

PAPAVERACEAE:

Sanguinaria canadensis, no fungus observed.

FUMARIACEAE:

Dicentra canadensis, no fungus observed.
Dicentra cucullaria, no fungus observed.

CRUCIFERAE:

Cardamine douglassii, no fungus observed.
Dentaria laciniata, no fungus observed.

PLATANACEAE:

Platanus occidentalis, endotrophic mycorhizas.

ROSACEAE:

Agrimonia mollis, endotrophic; vesicules present.

Crataegus crus-galli, endotrophic mycorhizas.

Crataegus mollis, endotrophic mycorhizas.

Geum canadense, endotrophic; fungus not abundant.

Prunus serotina, endotrophic mycorhizas.

Rubus occidentalis, endotrophic mycorhizas.

LEGUMINOSAE:

Cercis canadensis, endotrophic; one root shows perithecium containing large spiny two-celled spores.

Desmodium grandiflorum, endotrophic; fungus much disintegrated.

Gleditsia triacanthos, endotrophic mycorhizas.

Gymnocladus dioica, no fungus observed.

RUTACEAE:

Zanthoxylum americanum, no fungus observed.

EUPHORBIACEAE:

Acalypha virginica, endotrophic; fungus scarce.

LIMNANTHACEAE:

Floerkea proserpinacoides, no fungus observed.

CELASTRACEAE:

Celastrus scandens, endotrophic mycorhizas.

Evonymus atropurpureus, endotrophic mycorhizas.

ANACARDIACEAE:

Rhus toxicodendron, endotrophic mycorhizas.

STAPHYLEACEAE:

Staphylea trifolia, endotrophic mycorhizas.

ACERACEAE:

Acer negundo, endotrophic mycorhizas.

Acer saccharum, endotrophic mycorhizas.

Acer saccharinum, endotrophic mycorhizas.

SAPINDACEAE:

Aesculus glabra, endotrophic mycorhizas.

BALSAMINACEAE:

Impatiens biflora, endotrophic mycorhizas.

Impatiens pallida, endotrophic mycorhizas.

VITACEAE:

Psedera quinquefolia, endotrophic mycorhizas.

Vitis cordifolia, endotrophic mycorhizas.

TILIACEAE:

Tilia americana, ectotrophic mycorhizas.

VIOLACEAE:

Viola affinis, endotrophic; fungus scarce.

Viola scabriuscula, endotrophic; vesicules present.

ONAGRACEAE:

Circaea intermedia, no fungus observed.

Oenothera biennis, no fungus observed.

ARALIACEAE:

Panax quinquefolia, endotrophic mycorhizas.

UMBELLIFERAE:

Cryptotaenia canadensis, endotrophic mycorhizas.

Osmorhiza claytoni, no fungus observed.

Osmorhiza longistylis, endotrophic mycorhizas.

Sanicula canadensis, endotrophic mycorhizas.

CORNACEAE:

Cornus stolonifera, endotrophic mycorhizas.

PRIMULACEAE:

Steironema ciliatum, endotrophic; fungus scarce.

OLEACEAE:

Fraxinus americana, endotrophic mycorhizas.

Fraxinus pennsylvanica lanceolata, endotrophic mycorhizas.

Fraxinus quadrangulata, endotrophic; fungus scarce and much disintegrated.

ASCLEPIADACEAE:

Asclepias syriaca, endotrophic mycorhizas.

POLEMONIACEAE:

Phlox divaricata, no fungus observed.

HYDROPHYLLACEAE:

Hydrophyllum appendiculatum, no fungus observed.

Hydrophyllum canadense, no fungus observed.

Hydrophyllum virginianum, no fungus observed.

BORAGINACEAE:

Lappula virginiana, no fungus observed.

Mertensia virginica, no fungus observed.

VERBENACEAE:

Verbena urticaefolia, endotrophic; vesicules present.

LABIATAE:

Agastache nepetoides, endotrophic; fungus scarce.

Blephilia hirsuta, endotrophic; fungus disintegrated.

Leonurus cardiaca, no fungus observed.

Nepeta cataria, no fungus observed.

Nepeta hederacea, no fungus observed.

Prunella vulgaris, no fungus observed.

SOLANACEAE:

Solanum carolinense, no fungus observed.

SCROPHULARIACEAE:

Collinsia verna, no fungus observed.

Scrophularia marilandica, endotrophic; fungus disintegrated and not abundant.

Verbascum thapsus, no fungus observed.

Veronica virginica, no fungus observed.

BIGNONIACEAE:

Tecoma radicans, endotrophic mycorhizas.

ACANTHACEAE:

Ruellia strepens, endotrophic mycorhizas.

PHRYMACEAE:

Phryma leptostachya, no fungus observed.

RUBIACEAE:

Galium aparine, no fungus observed; many cortical cells contain coccus-like bacteria.

Galium concinnum, endotrophic; fungus very scarce.

CAPRIFOLIACEAE:

Sambucus canadensis, endotrophic mycorhizas.

CAMPANULACEAE:

Campanula americana, endotrophic mycorhizas; vesicules present.

LOBELIACEAE:

Lobelia inflata, endotrophic; fungus very scarce.

Lobelia siphilitica, no fungus observed.

COMPOSITAE:

Actinomeris alternifolia, endotrophic mycorhizas.

Ambrosia artemisiifolia, endotrophic mycorhizas.

Ambrosia trifida, endotrophic mycorhizas.

Aster ericoides, endotrophic mycorhizas.
Aster sagittifolius, endotrophic; much of the fungus disintegrated.
Aster shortii, endotrophic; fungus very scarce.
Bidens vulgata, endotrophic mycorhizas.
Cacalia reniformis, endotrophic; fungus very scarce.
Erigeron canadensis, no fungus observed.
Erigeron philadelphicus, no fungus observed.
Eupatorium purpureum, endotrophic; fungus disintegrated.
Eupatorium urticaefolium, no fungus observed.
Helianthus decapetalus, endotrophic mycorhizas.
Lactuca floridana, endotrophic; fungus disintegrated.
Lactuca scariola, endotrophic; fungus very scarce.
Polymnia canadensis, no fungus observed.
Rudbeckia laciniata, endotrophic mycorhizas.
Rudbeckia triloba, endotrophic mycorhizas.
Solidago rugosa, endotrophic mycorhizas.
Taraxacum officinale, endotrophic mycorhizas.
Vernonia illinoiensis, endotrophic; fungus disintegrated.

This list contains 145 species distributed among 114 genera and 60 families. Mycorhizal fungi were found in 93 species, 76 genera, and 43 families; while in 52 species, 38 genera, and 17 families no mycorhizal fungi were observed. In other words, either ectotrophic or endotrophic mycorhizas were found in 64.1 per cent of the species, 66.6 per cent of the genera, and 71.6 per cent of the families. It is quite possible if not probable, of course, that more extensive observations would materially decrease the percentage of negative results. Perhaps the most significant fact brought out by these observations, however, is the large percentage of plants in this forest that harbor mycorhizal fungi, which reemphasizes the great importance of a clearer understanding of mycorhizal phenomena than has heretofore been possible.

Of the 93 species that were found to possess mycorhizas, only 8 (all of which are trees) were ectotrophic; while 86 were endotrophic, *Carya cordiformis* showing both mycorhizal types. Endotrophic mycorhizas in species of *Carya* are here reported for the first time, although the ectotrophic type is common in this genus and the endotrophic type is well known in the closely related genus *Juglans*.

Apparently there are very few woody plants that do not at times harbor mycorhizal fungi of one or more kinds. The only woody plants in this forest on which no mycorhizas were found are *Ulmus americana*, *Benzoin melissaefolium*, *Gymnocladus dioica*, and *Zanthoxylum americanum;* and one of these, *Ulmus americana*, has been found with endotrophic mycorhizas in other habitats. It is interesting to note, too, that in some of the larger families, such as Rosaceae and Compositae, mycorhizas are common; while in Labiatae and Scrophulariaceae they are relatively uncommon.

Nearly all of the endophytic fungi observed in this study appear to be phycomycetous. Basidiomycetous mycelia showing septa and clamp connections were found in *Aplectrum hyemale*, *Carya laciniosa*, and *Humulus lupulus;* but in all cases they appeared to be casual parasitic invaders rather than true mycorhizal fungi. Entirely comparable with these basidiomycetous parasites would be the bacteria that were observed in the cortical cells of *Galium aparine* and the perithecia-forming fungus in one root of *Cercis canadensis*.

In the case of practically every endotrophic mycorhizal plant here reported, nothing definite is known concerning its mycorhizal relations except that it has mycorhizas. They thus furnish a very fertile field for investigation. In some the endotrophic fungus is rare while in others it is practically always present and usually abundant. In the maples, for example, the fungus is almost always to be found in the cortical cells of beadlike swellings of the small rootlets. It is hoped that opportunity for further investigation of this particular genus may be forthcoming.

At present there is absolutely no evidence that any of the higher plants here considered are benefited by the presence of mycorhizal fungi. It is possible that in some of these cases the relation between fungus and higher plant is reciprocal, as it has been found to be in the orchid family and in some members of the heath family; but up to the present we have no basis for believing that this is true. In many cases the fungus mycelium appears much disintegrated, but in no case has evidence of the actual digestion of fungus filaments by the host cells been seen, as reported in the preceding families; and no attempts have yet been made to determine whether seedlings of any of these plants may be grown under sterile conditions in the

absence of fungi. On the other hand, it is well known that the aerial parts of plants are subjected to the attacks of a variety of relatively harmless parasites, such as gall-forming insects, leaf-spot fungi, etc., and there is no reason for supposing that the subterranean parts are exempt from similar attacks. For the present, therefore, it is believed that all of the mycorhizas reported in this paper should be classified under antagonistic nutritive conjunctive symbiosis, the fungus being parasitic on the higher plant.

UNIVERSITY OF ILLINOIS
URBANA, ILL.

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EFFECT OF ALCOHOL ON CELLS OF NITELLA FLEXILIS

P. A. DAVIES

(WITH ONE FIGURE)

Introduction

An initial rise in the rate of CO_2 production[1] is induced by the action of ethyl alcohol on cells of *Nitella flexilis*. Such a rise has been observed by others using different substances. IRVING (7) observed a rise when young shoots of *Hordeum vulgare* were subjected to low concentrations of chloroform, but with high concentrations no rise occurred. HAAS (4, 5, 6), using alcohol, acetone, formaldehyde, and ethyl chloride on *Laminaria* tissue, and GUSTAFSON (3), using formaldehyde, ether, and acetone on *Aspergillus niger*, found that when the concentrations were high enough to produce an effect, there was an increase, followed by a decrease, in the rate of CO_2 production. HAAS (4, 5) found the rate to be higher than the normal even after the tissue was killed. BROOKS (1) found an enormous rise (50 times the normal) when *Bacillus subtilis* was treated with 7.3 per cent ether solution, but no rise with high concentrations. RAY (13) found that subjecting *Ulva* tissue to 0.25 per cent chloroform solution gave an initial rise, but above 0.5 per cent only a decrease from the normal was observed. The writer's results with alcohol are very similar to those of HAAS and GUSTAFSON, but differ from those of IRVING, BROOKS, and RAY, in that an initial rise occurred with all concentrations used.

This preliminary paper is concerned with a brief explanation of the cause of the relative heights of the initial rises. In all experiments a normal rate of CO_2 production was established (taken as 100 per cent) before the alcohol was added.

Discussion of results

Fig. 1 shows the relative heights in the initial rate of CO_2 production induced by the action of different concentration of alcohol.

[1] For apparatus used in CO_2 determinations, see OSTERHOUT (11). All experiments were conducted at 30° C.

Comparing the initial rises, the 20 per cent (by volume) solution caused the highest initial rise, followed by the 10, 30, 40, 60, and 95 per cent solutions respectively. The drop of the initial rises below the normal rate was most rapid in the 95 per cent solutions, followed by the 60, 40, 30, 20, and 10 per cent solutions respectively; al-

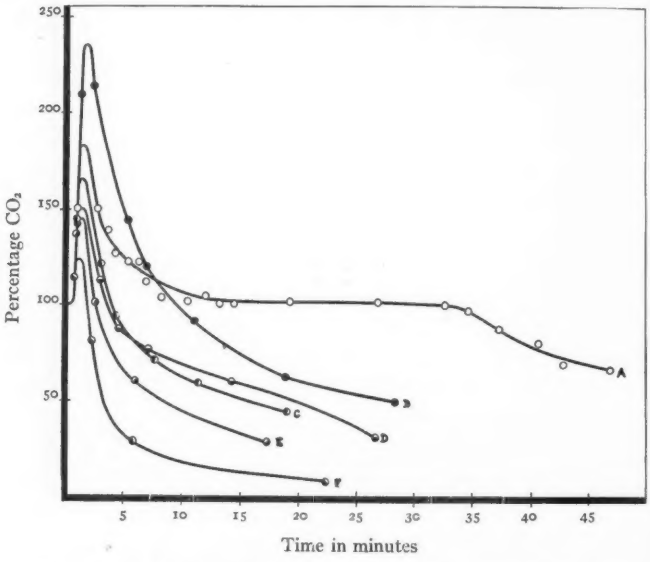

FIG. 1.—Rate of CO_2 production from cells of *Nitella flexilis* in different concentrations of ethyl alcohol, as percentages of normal rates: *A*, effect of 10 per cent (by volume) solution; *B*, 20 per cent; *C*, 30 per cent; *D*, 40 per cent; *E*, 60 per cent; *F*, 95 per cent. Each curve represents a single experiment best illustrating the average course of all (3 to 13) experiments.

though in the 10 per cent solution the rate of CO_2 production did not fall below the normal rate until about 35 minutes after the solution was added.

The question might arise as to whether the relative increases may not be due to the coefficient of CO_2 absorption by the different concentrations of alcohol. If the coefficient of CO_2 absorption by alcohol alone is considered, we should expect to find the highest initial rise in a concentration greater than 20 per cent. MÜLLER (10) found the

lowest coefficient of CO_2 absorption by alcohol at approximately 20° C. in a solution of 28.46 per cent by weight (36.25 per cent by volume). LUBARSCH (9) confirmed MÜLLER's results by finding the lowest coefficient of CO_2 absorption in a 28.5 per cent solution; so it appears that the highest initial rate obtained in the 20 per cent solution is independent of the CO_2 absorption by the alcohol, and must be due to the direct action of the alcohol on the structures of the cell.

As to the source of the CO_2, BROOKS believes that the probable source is CO_2 previously stored in the cell, either as CO_2 or in the form of carbonates or bicarbonates. The writer's work seems to show that the chief source of CO_2 is CO_2 dissolved, under tension, in the cell sap and not in the form of carbonates or bicarbonates. When extracted cell sap of *Nitella*[2] was used an initial rise was secured when distilled water was added. When new extractions of the cell sap were taken and CO_2-free air allowed to bubble through them for 10 minutes, when tested with distilled water or dilute hydrochloric acid, no initial rise was secured; in fact no measurable amount of CO_2 was secured. In order to check these results, potato sap known to contain dissolved CO_2 was tested, and similar results were obtained. The effect of the distilled water must be on the CO_2 equilibrium of the extracted saps. That CO_2 is held in the cell sap under considerable tension is shown by CROZIER (2) and by OSTERHOUT and DORCAS (12) with cells of *Valonia macrophysa*. They found that the sap is a poor buffer, CROZIER finding that 1–2 cc. of sea water was sufficient to change 10 cc. of the sap from pH 5.9 (average pH of the cell sap) to 7.0. IRWIN (8) gives the pH value of *Nitella* sap as 5.6; a greater internal C_H than was found for *Valonia*. The sudden increase in CO_2 production when distilled water was added to *Nitella* sap, with a rapid fall below the normal, and the fact that no production of CO_2 can be observed after CO_2-free air has been bubbled through the cell sap for 10 minutes, indicate, as has been shown for *Valonia*, that the pH value of the sap must be due chiefly to CO_2 dissolved in it. RAY thinks that the initial rise is due to the fact that the reagent (chloroform) reacts directly with the oxidase

[2] The cells were washed several times in distilled water and squeezed between several layers of neutralized and sterilized cheesecloth, and the sap was placed in the apparatus in an unfiltered condition.

system, either as a catalyzer or by the formation of a loose compound with some portion of this system. The writer obtained an initial rise when normal cells of *Nitella* were treated with distilled water, as when (as has already been explained) distilled water was added to the extracted saps of *Nitella* and potato. It is hard to believe that water could produce such a rapid production of CO_2 by catalysis, for in the case of potato sap it was diluted with distilled water before being placed in the apparatus. The action of the water on the saps must be due simply to the upsetting of the CO_2 equilibrium to such an extent that CO_2 is released. The action of distilled water on the normal cells must be such as to produce a sudden change in the plasma membranes, so that CO_2, where it is held under considerable tension, is rapidly given off for a short time.

The action of the alcohol depends on its concentration: in the 10 per cent solution it did not penetrate[3] the cell structures rapidly enough to cause a rapid release of CO_2 induced by the 20 per cent solution; in the 30, 40, 60, and 95 per cent solutions the alcohol caused a change of the cell structures to such an extent that CO_2 was released slowly from the irreversibly injured cells and was taken up by the solution, as it escaped, according to the coefficient of CO_2 absorption by the solutions; and in the 20 per cent solution the alcohol caused an intermediate effect, a rapid penetration without a rapid change of the cell structures, allowing a more rapid outflow of CO_2.

Summary

1. The relative rises in the initial rates of CO_2 production are due to the direct action of the different concentrations of ethyl alcohol on the complex structures of the cell, which control the internal CO_2 tension.

2. The 20 per cent (by volume) solution caused the highest initial rise, followed by the 10, 30, 40, 60, and 95 per cent solutions respectively.

3. The drop of the initial rises below the normal rates was most rapid in the 95 per cent solution, followed by the 60, 40, 30, 20, and 10 per cent solutions respectively.

[3] Irreversible injury, considered to have taken place when the rigidity of the cell was lost, did not occur until about 33 minutes and 58 seconds (average time) after the alcohol was added.

4. The highest initial rise in the 20 per cent solution is produced by a rapid penetration of the alcohol without a rapid change in the cell structure, allowing a rapid outflow of CO_2.

UNIVERSITY OF LOUISVILLE
LOUISVILLE, KY.

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BRIEFER ARTICLES

THE MULTIPLE-SEEDED XANTHIUM

CHARLES A. SHULL

(WITH ONE FIGURE)

The discovery of another native specimen of the multiple-seeded *Xanthium*, which is tentatively known as *Xanthium chinense globuliforme*, in a locality far removed from the previously recorded localities for this form, emphasizes the sporadic occurrence of this peculiar type of cocklebur. This specimen, which is illustrated in fig. 1, was found by Mr. GEO. M. CHASE, who lives about 6 miles southeast of Allen, Nebraska, a short distance west of Sioux City. The plant was found growing in a cornfield, among many other specimens of *Xanthium* and *Helianthus*. It attracted attention because it was so different from its surrounding plants, and was sent to Professor W. W. BURR of the University of Nebraska for identification. Through the kindness of Professors BURR and N. F. PETERSON, who recognized its similarity to the previously reported multiple-seeded forms, it was sent to the writer for examination.

The photograph is too small to show the characteristic structure of the bur, unless examined with a hand lens; but it is quite similar to that of those previously described.[1,2,3] This particular plant is over 4 feet tall, and shows evidence of great vegetative vigor. It is completely sterile. The burs are short and rather deeply cleft. While a few shriveled ovaries can be found in the largest burs, none of them contains even partially developed embryos. The stem is very strongly fasciated, as is readily seen in the figure. In its tendency to complete sterility, in its vegetative vigor, in its strongly fasciated stem, it resembles many of the specimens of multiple-seeded *Xanthium* which have been grown from seed in the garden of the Hull Botanical Laboratory.

In a recent paper[3] the various possible interpretations of this

[1] SHULL, C. A., An interesting modification in *Xanthium*. Amer. Jour. Bot. **3**:40–43. 1917.

[2] ———, Multiple-seeded burs of *Xanthium*. Science **58**:145–146. 1923.

[3] ———, Nature of the multiple-seeded *Xanthium*. BOT. GAZ. **83**:385–398. 1927.

type of *Xanthium* have been summarized. Reversion to remote ancestry, mutation, fasciation, and hybridization are the main possibilities. Similar "reversions" have been reported among other genera of the Compositae, and fasciation is rather readily induced in this family. After careful study of the behavior of the plants through a number of generations, it seemed more likely that the freak multiple-seeded condition is a consequence of fasciation, rather than of hybridization or reversion. Yet there are a number of facts which deserve consideration.

In the second generation offspring of the specimen found in Kansas City in 1925, there was great variability in size. There was a suggestion of segregation in the differences noted in the size and vigor of growth of specimens during the summer of 1927. Yet there has never been any clear cut segregation in regard to bur character, such as one might expect if the multiple-seeded forms are hybrids. As these specimens were started under rather poor greenhouse conditions, which may have affected their vigor after transplanting to the garden, the experiment is to be repeated with seeds planted in the garden at the normal season of germination.

FIG. 1.—Sterile native specimen of multiple-seeded *Xanthium*

An interesting observation has been made by Miss SYMONS,[4] which is worthy of serious consideration. She reports cases of bur character segregations, as for instance, burs of *X. pennsylvanicum* giving in the next generation both *chinense* and *pennsylvanicum*. She describes these burs very briefly, as follows:

[4] SYMONS, JENNIE L., Studies in the genus *Xanthium*. BOT. GAZ. 82:121–147. 1926.

Their fruits were small, with few prickles, and characteristic wide open beaks, which were purple-tipped when immature. The latter, usually indicative of the number of seeds in the bur, varied in number from 2 to 7. The formation of more than the normal number of beaks and seeds was especially characteristic of these *X. chinense* plants, although it occurred to some extent in all plants of this lot.

This is the only evidence we have that seems to link multiple-seededness with hybridization.

Miss SYMONS says nothing about the bearing which this observation has upon the nature of the naturally occurring multiple-seeded specimens, but it is very suggestive, and deserves much careful study. She proceeded to cross various species, particularly *X. italicum* and *X. curvescens* with each other, and with *X. inflexum* and *X. pennsylvanicum*, and demonstrated the possibility of such crosses. Her tests of the possibility of parthenogenesis agree with some results obtained by the writer at Johns Hopkins University in 1923. There is apparently no parthenogenesis, and cross fertilization is not abundant between specimens isolated by a distance of 50 feet or more from other plants of the same species.

Examination of the burs produced by the hybrids obtained by Miss SYMONS fails to reveal anything like the burs which have been found on these multiple-seeded specimens found occurring sporadically in nature. Whether succeeding generations from these hybrids would show the same type of multiple-seeded condition is problematical; but the fact that some multiple-seeded specimens of *X. chinense* were observed in her studies, whether of the same type as the native burs or not, should lead to a thorough testing of species crosses, to determine whether multiple-seeded burs like those in nature can be produced by crossing the proper species. Granting the possibility that these sporadic multiple-seeded forms may be due to rare hybridizations, yet we have never noted any segregation of bur types in successive generations, and fasciation is practically universal in the native specimens and their offspring. This has not been reported to be the case with the crosses so far achieved, but should be looked for in all cases of known hybrids. The phenomena may be restricted to certain species crosses, and fail to develop in other cases of hybridization.

UNIVERSITY OF CHICAGO

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CURRENT LITERATURE

BOOK REVIEWS

Leaf miners

A volume has been prepared by NEEDHAM, FROST, and TOTHILL[1] which will be of interest and use to botanists, to lovers of plants, and to plant pathologists as well as to entomologists.

Chapters I and II are of general biological interest, and afford pleasant reading. Phrases are used in places for which a botanist would take a fellow botanist to task; possibly the entomologist should be given greater freedom. There is slight justification for referring to leaf vessels as "channels of circulation," however, or for grouping everything between the lower and upper epidermis, veins included, as "mesophyll." Various orders and families of insects which play a rôle as leaf miners are discussed in the following twelve chapters. Chapters XV and XVI will prove especially welcome to anyone not a professional entomologist. The former consists of a list of leaf-mining insects, while the latter contains a list of hosts of leaf miners and an extensive bibliography. In this list plants are given under both scientific and common names, when they have the latter. There are listed 422 hosts. For any one primarily interested in plants, this list is the most important feature of the book.—G. K. K. LINK.

Truck-crop plants

A book has been prepared by JONES and ROSA[2] which will serve as a very general reference volume for those interested in the plants which are classed as truck-crops. There is a brief discussion of the taxonomy, histology, morphology, physiology, ecology, pathology of non-parasitic and parasitic origin, and genetics of these plants. In addition, some attention is devoted to the breeding, cultural, and marketing practices associated with these crops. In presenting these data, the authors have succeeded in their task of preparing a survey of present information on truck-crop plants in which the plant rather than "certain practices or phenomena" is made the basis for discussion.—G. K. K. LINK.

NOTES FOR STUDENTS

Photosynthesis.—DANGEARD[3] has recently devoted a whole volume of his botanical journal to the publication of the results of his work on photosynthesis scattered over some twenty years. Ignored by his compatriots, although

[1] NEEDHAM, J. G., FROST, S. F., and TOTHILL, BEATRICE H., Leaf mining insects. 8vo. viii+351. *figs. 91.* Baltimore: Williams and Wilkins Co., 1928.

[2] JONES, H. A., and ROSA, J. T., Truck-crop plants. 8vo. xiv+538. *figs. 98.* New York: McGraw Hill Book Co. 1928.

[3] DANGEARD, P. A., Recherches sur l'assimilation chlorophyllienne et les questions qui s'y rattachent. Botaniste **19**(1/6):1–397. 1928.

doing the best work in this field which France has produced, it is not surprising that his name is little known as a student of this problem. His papers hitherto have been rather brief notes of progress, often in somewhat obscure journals and always with the irritating lack of details so customary in French scientific papers. Even the two recent exhaustive monographs on photosynthesis by STILES and by SPOEHR fail to make any reference to DANGEARD'S work, although it is cited in several of the papers they review. Because of this curious neglect, and because the work reported seems unusually interesting, it is called to the attention of plant physiologists in some detail.

It is unfortunate that most of the work now reported was done before the war, although it has been confirmed by recent repetition. Had it been published in full then, it would undoubtedly have been acclaimed loudly; now much of his work has been better done by others, especially as regards quantitative effects of light. But DANGEARD has studied particularly the qualitative effects, and has much that is still worth reading. His "new method," published in 1909, is still unadopted by anyone else. It is based on the use of cultures of nonmotile, unicellular green algae, especially *Chlorella* and *Scenedesmus*. These algae in an inorganic nutrient medium are quite unable to multiply appreciably in the dark, whereas in the light they multiply rapidly. Such a culture, placed behind a series of ray filters or in a spectrum, will cover the vessel wall in regions where photosynthesis can take place, but fail to multiply and become visible in regions where it cannot occur. Furthermore, such cultures give off bubbles of oxygen which are readily counted (1–400 per m.), and the rate of bubble production is a much safer criterion of photosynthetic activity than in the case of *Elodea*. There is no inactive tissue and no internal aerating system to hold nitrogen, as in *Elodea*, and changes in light intensity are followed very quickly by changes in bubble rate, so that a new equilibrium is established in 1–3 minutes. These algal cultures are easily maintained in quantity, and cultures giving comparable results readily selected, so that the material can be standardized. Although WARBURG has used these algae for his quantitative studies of photosynthetic efficiency, nobody but DANGEARD has used them for the ordinary studies (and laboratory experiments!) on photosynthesis. The reviewer believes that here is a tool to our hand which may give valuable service.

The historical introduction covers 63 pages, and is a well tempered but penetrating critique of the work up to date, especially with respect to the qualitative effect of light. The only omission noted is of the work of WARBURG and NEGELEIN, which did not appear in a botanical journal. Particular attention is given to the errors involved in studies using ray filters, such as $CuSO_4$ solution, and the necessity pointed out of determining spectroscopically the wave lengths which pass. WARBURG and NEGELEIN did this, and tried to correct for undesired transmission; but most workers have failed to note the parasitic radiations. The fallacy of WURMSER'S well known and oft criticized anomalous results is declared to be due to his green filter passing red rays of great photosynthetic activity.

The author has attacked the problem of the relation between wave length and photosynthesis with both ray filters and spectra. The Wratten series of seven monochromatic gelatin filters was used principally for the first method, and each filter was analyzed with the spectroscope before and after use, as they were found to change in range of transmission with use. For spectral work three glass prism and one quartz system spectrographs were employed. The latter gave an approximately normal spectrum of considerable intensity throughout, dispersion in the violet being less than twice that in the red. A 1 mm. slit was used. The light source for spectral work was usually an electric filament lamp.

Whether with ray filters or with spectra, there was algal growth and bubble production only at the position of the absorption bands of chlorophyll, and the amount of growth or bubbles was proportional to the degree of absorption. The green covering on the walls of the culture used in the spectrum was in bands agreeing with the absorption bands. This was true of all absorption bands save the very strong ones in the blue-violet, where neither growth nor bubble production was appreciable, even after several days' illumination. Using the filters with very intense light, as direct insolation, there might be considerable activity in regions not ordinarily active, as in green and blue-violet, and the author produces considerable evidence for his view that this is almost wholly accounted for by the parasitic red rays passed then. It is unfortunate that no measurements were made of the energy distribution of the spectrum of the electric lamp used, but sunlight studies confirmed those with the artificial light source. Evidence is presented that multiplication of algae in the B–C region of the spectrum is 98 per cent as rapid as in the full light of the source.

An ingenious method of demonstrating the position of the wave lengths responsible for chlorophyll decomposition has also been proposed by DANGEARD. Equal quantities of strong alcoholic extract of the pigment and of alcoholic solution of collodion are mixed and evaporated in a thin layer on a glass plate. This is placed in the spectrum and the places noted where green disappears. Decomposition of chlorophyll occurs in bands corresponding to those of absorption, except that as in photosynthesis the blue-violet seems little active.

Using this same method for mixtures of chlorophyll and other pigments with dyes, the author has studied photosensitization. His conclusions in this field are far from being as novel as he seems to think them, but his method and some of his results are of great interest. Traces of chlorophyll cause rapid decomposition of these dyes in the B–C region, where they do not absorb at all, and traces of bacteriochlorin have the same effect as chlorophyll, except that decomposition is first in the region of its absorption bands in the far and infra red. On the other hand, the carotinoids and bacteriopurpurin have no such photosensitizing effect. The obvious functional correlations are very plausible at least, and are supported by other evidence. Incidentally, the author suggests that the function of the carotinoids is to protect chlorophyll from decomposition in light by absorbing all the adjacent oxygen! Later he admits the possibility of the protecting screen theory of IVANOWSKI.

A further contribution is made to the problem of the wave length used by algae of different color. Blue-green algae and diatoms showed their principal growth bands, both in spectra and behind filters, in the same region as green algae, with an additional band in the far red for the blue-green algae, which have an absorption band at about 720 $\mu\mu$. The sulphur bacteria developed only behind filters passing the red and infra red, which is in agreement with the position of their absorption bands. Because of their motility they could be used to form growth bands in the spectrum, but they congregate in a spectrum to form "fixation" bands, as do motile blue-green algae, corresponding to the absorption bands in position.

Less happy are the conclusions regarding the ability of deep water algae to utilize the penetrating yellow-green rays by reason of having chlorophyll with stronger absorption in this region than is exhibited by chlorophyll of land and shallow water plants. The evidence brought forward is very faulty, and in the few cases where strong absorption by bands in the yellow-green is found for chlorophyll from brown and red algae, it is evident that concentration alone is the explanation. There is even reported extraction of a chlorophyll which when separated from the carotinoids had no absorption in the blue-violet, and TSWETT is erroneously cited as having found that his chlorophyllin α had very little absorption there. The absorption bands for phycoerythrin are not in agreement with those found by others. Altogether, this chapter of the work is about the least satisfactory.

The author's study of chlorophyll development in the nearly normal spectrum shows a sharp boundary at 680–690 $\mu\mu$, whereas greening is found in varying degrees down to 440 $\mu\mu$, with blanched leaves of endive. The maximum development is in the red between B and C, with a weak secondary in the blue-violet. Really more critical is the study using seedlings of *Lepidium* germinated in close ranks in the spectrum. Here the red limit and the maximum are the same as for the leaf, but the lower limit is somewhere near 570 $\mu\mu$. Spectroscopic analysis of the extract from seedlings, however, showed traces of green from the seedlings in wave lengths below this, and the limit is not really determined absolutely.

SAYRE[4] recently studied this question, using ray filters, and reports the same red limit, but no limit on the blue side down as far as 300 $\mu\mu$, if enough energy is supplied. Actually he does not prove that wave lengths shorter than 400 $\mu\mu$ are effective, and in the only case in which waves up to 500 $\mu\mu$ were not transmitted, only traces of green were recorded. The question of the ultra limit of chlorophyll formation is still open. Of great interest is the purely accidental correlative finding of the author that the lower limit of visible chlorophyll development, about 510 $\mu\mu$, is the upper limit of phototropic response, which continues strong through the more refrangible part of the spectrum to its limit, here 320 $\mu\mu$.

[4] SAYRE, J. D., The development of chlorophyll in seedlings in different ranges of wave lengths of light. Plant Physiol. 3:71–78. 1928.

The reviewer has only called attention to some of the more notable features of this very interesting and long neglected work. He believes that the author is correct in the attitude expressed in the preface, that the new methods suggested are more valuable than the data presented. The work is marred by such misprints as invariably 0.02 cm. and 0.001 mm. where obviously 2 cm. and 1 mm. are meant. And the author cannot realize that quantitative measurements of chlorophyll absorption have shown that the absorption bands are only places of local maximum absorption, with the region between bands absorbing only less strongly than the weakest band. Nowhere, also, is any measurement made of light intensity, nor is it intimated that the author realizes that the various ray filters have quite different transmission intensities. Occasionally rather definite conclusions are drawn which are invalid until intensity differences are studied and eliminated. On the other hand, the author has made the bubble-counting method productive of very significant results, and has added several new schemes to those already available for its use. Students of photosynthesis can no longer afford to ignore DANGEARD's work.—H. S. WOLFE.

Maine vegetation.—Mount Desert, a rugged and rocky island off the coast of Maine, appears to offer many problems of peculiar ecologic interest. Situated on the tension line between the conifer forests of the north and the deciduous forests of New England, there are within its 100 square miles a variety of forest types ranging from those of northern Canada to those of New Jersey. Among the well developed forest associations are those characterized respectively by spruce, fir, northern hardwoods, white pine, white cedar (*Thuja*), mixed conifers, and pitch pine (*Pinus rigida*).

The environmental factors which control this mosaic of vegetation have recently attracted the attention of MOORE and TAYLOR,[5] who have studied the topography, soil, and climate, as well as the vegetation. Among their data those regarding evaporation are most extensive and most interesting. Pairs of stations established both within and without four of the principal forest types, and maintained throughout the growing seasons of 1921, 1922, and 1923, exhibited remarkable agreement. They plainly demonstrated that not only is the spruce forest the most mesophytic and the *Pinus rigida* association the most xerophytic, but the character of the open sites varies with that of the forest interiors. In other words, the most northern forest type has taken possession of the most mesophytic situations, and the most southern type has persisted in the most arid.

Throughout all the habitats the rates of evaporation are surprisingly high. This is notably shown in the *Pinus rigida* type, where the evaporation is higher than within the same forest much farther south on Long Island. In addition, the average rate in the most mesophytic forest seems to be nearly double that

[5] MOORE, BARRINGTON, and TAYLOR, NORMAN, Vegetation of Mount Desert Island, Maine, and its environment. Brooklyn Bot. Gard. Memoirs 3:1–151. *figs.* 26. *Map.* 1927.

found by the reviewer in somewhat similar forests in the Chicago region.[6] These high rates seem to be due to the prevalence of dry southwest winds.

The soil temperatures, taken throughout the three seasons at 6 and 18 inches below the surface, show little difference between the various associations. The *Pinus rigida* has slightly the warmest soil, probably due to its drier character quite as much as to its exposure. It would have been very interesting to have had the range of soil moisture for the three seasons. Without such data the soil analyses, with wilting coefficients and water-holding capacity, have relatively little significance.

In addition to data on environment, the various forest associations are carefully described and their successional relations investigated. Two climax forests are distinguished, the one dominated by spruce (*Picea canadensis* and *P. rubra*), and the other by a mixture of spruce and northern hardwoods, among which sugar maple (*Acer saccharum*) is conspicuous. In the study of the successional relations, the establishment of the tree species does not seem to have received the attention devoted to the herbaceous and shrubby forms.

The study as a whole is to be highly commended for its general excellence, its organization, and its elucidation by photographs, diagrams, and graphs.—G. D. FULLER.

Regulation of substratum acidity by species of Sphagnum.—It has been the prevalent opinion that *Sphagna* cannot tolerate lime. STELMACH[7] undertook to investigate the two species, *S. recurvum* and *S. cymbifolium*, by culture methods with regard to the effect (1) of the Ca ion on the development of the moss plants, (2) of the alkaline nature of the substratum on development, and (3) of the moss plants in neutralizing alkaline soil and acidifying acid soil. By using Knopp's calcareous nutrient and calcium-free nutrient solutions, it was found that calcium has no toxic effect in the case of either of the two species tested. While concentrations of 0.2 gm. of Na_2CO_3 and $CaCO_3$ respectively per liter of solution caused death of all cultures within four weeks, plants of both species thrived in a solution containing 0.125 gm. of $CaCO_3$ per liter. It was found that the solutions, after three weeks, gave a neutral test with litmus and not an alkaline test as at the beginning. The neutralization was supposed to be due to the effect of the liberated "wooer" acids. The increase of H-ions hastened development only within certain limits. *S. recurvum* lived within the limits pH 6.8–5.2, with 5.8 as the optimum, while *S. cymbifolium* lived within the limits pH 6.0–4.8, with an optimum of pH 5.4. Both species acidified the weakly alkaline solution and made it fit for their own development. *S. recurvum* defended itself against overacidity by weakening it; *S. cymbifolium* increased the acidity of the acid substratum and died on account of excess.—J. ISENBARGER.

[6] BOT. GAZ. 58:217. 1914.

[7] STELMACH, ——, Die Regulation der Substratacidität durch zwei Torfmoose *Sphagnum recurvum* and *S. cymbifolium*). Extrait du Bulletin l'Academie Polonaise et des Lettres Classe des Sciences Mathematiques et Naturelles. Nat. Ser. B. 1926.